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Improving quality

**Edited by Douglas B. MacDougall**



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## **Colour in food**

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# **Colour in food**

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**Edited by  
Douglas B. MacDougall**



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# 1

## Introduction

**D. B. MacDougall, formerly of the University of Reading**

Of our five senses, vision, hearing, touch, taste and smell, the sense of vision plays a continuous and important role in daily experience. The world around us is perceived as being both in motion and coloured. From waking, throughout the day until sleep, we rely on our eyes, whether consciously or otherwise, to provide information for the many routine decisions confronting us. Our response to the myriad of visual signals causes us to accept or reject the consequences of this information on the basis of their importance and immediacy of action demanded. For example, recognition of people, choice of apparel to wear, warnings from traffic lights, interpretation of the weather, aesthetic pleasure in viewing art and, especially within the subject matter of this book, the selection of our daily food. The purchase of food and the processes of cooking and consumption, for most people, require decisions and actions leading to acceptance or rejection on aspects of the food's perceived quality. The appearance of the food is paramount in this process of human choice for selection and eating.

Our choice of food is governed by many factors, the principal of these being availability. In affluent societies, where there is abundant supply and variety of food items, the choice presented in the modern supermarket is immense. Supermarkets are likely, at any time, to stock several thousand items. Many of these will be different in type but others will be of a similar nature although supplied by a variety of manufacturers. Perusal of any supermarket shelf or refrigerated or frozen display cabinet will present the consumer with a decision process of what to purchase and for what purpose. The mechanism of human behaviour in this process of selection and purchase is the subject of several recent books (Marshall 1995; Meiselman and MacFie 1996; Mowen and Minor 1998; Sheth *et al.* 1999). High on the list of factors contributing to this process are the influences of the colour appearance of the food or food package and the ambience of the display surroundings (Cardello 1994). Success in the food



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industry depends on supplying consumers with what they want and doing it attractively, consistently and safely with consequential long-term consumer satisfaction leading to repeat purchase.

The term 'food' covers an enormous range of items and products. If food is defined as that which is eaten for our nutrition and pleasure, its variety and type takes many forms. These may be broken down essentially into fresh and raw produce, e.g., fresh vegetables, fruit, meat and fish; into dairy products, e.g., milk, butter, cheese, yoghurt, etc. and baked and cooked products, e.g., bread, biscuits and prepared meals. In addition to the basic materials of food supply there is an increasing demand for ready-prepared meals with their many components. Coupled with the range of raw materials are the processes that are used to produce, alter and preserve food. These include dehydration, canning, refrigeration, freezing and storage, all of which induce changes to the food, considered either advantageous, e.g., baking bread, or disadvantageous, e.g., the induction of unwanted browning, loss of pigment concentration and changes in the food's structure that reduce its attractiveness. During all these procedures, where colour and appearance alter, consumers' reactions to the product are likely to be affected.

Research into the colour of food, its chemistry and the factors that alter it, has been and continues to be the subject of a significant part of food research literature. The contents of any leading food journal are likely to have articles directly focused on the colour of the food or procedures where colour measurement has been used as a tool to follow its change or its relationship to other food qualities. For example, the recently issued CD, containing the text and abstracts of the *Journal of Food Science* (CD-ROM 2001), shows that the number of papers in the last five years in which food colour is a major topic is in the region of at least one research paper per issue. The colour measurement of food, in many instances, is accomplished with an understanding of the procedures involved but it is also clear that many food colour measurements are done without full comprehension of what colour actually is. Evidence for this can be seen in the way colour data is interpreted. Important in this lack of understanding is the fact that colour is usually assumed to be a property of the material whereas it is in reality the response of human beings to the visual signals generated by light on the product.

Over the past forty years, several important books have been published that have had the colour of food as their main objective. The earliest of these, from the 1960s and 1970s, succeeded in introducing the food industry to the scientific principles of instrumental colorimetry as related to food (Francis and Clydesdale 1975; MacKinney and Little 1962). The most up-to-date colour instrumentation at that time was the Hunterlab series of colour difference instruments using the Lab square root uniform colour scales developed in the period between 1950 and 1958 (Hunter and Harold 1987). These instruments had the significant advantage that the opponent colour scale differences could be computed and read directly from the instrument. Much has happened since then in both the understanding of human visual perception and the technology of colour measurement, for example, the use of the computer allows the technologist to obtain colour

information in the more precise cube root CIELAB  $L^*a^*b^*$  colour scales. A more recent book dealing with the colour of food is concerned with evaluating food colours and their relationships to the food's appearance (Hutchings 1999).

A major objective of this book is to relate the psychological processes involved in the human perception of colour and its associated appearance qualities with the many components and variables encountered in the visual judgement of foods within their contexts. This book is divided into two major parts, the first dealing with the perception of appearance and measurement of the colour of food and the second on colour control in food.

The first Chapter in Part I (Chapter 2) by J. B. Hutchings, on the perception and sensory assessment of colour, introduces the concept of the total appearance of food of which the perception of colour is one of many components. These components include the consequences of identification of the visual aspects of food on its potential eating quality which, in turn, leads the consumer to choice and acceptance. Visual assessment of the food presents the observer with the challenge to estimate the potential flavour and texture of the food and the satisfaction to be expected. Factors affecting total appearance are numerous and although colour is of major importance, other less easily quantifiable influences affect consumer reaction to product appearance. These include effects of the environment, e.g., lighting and display, learned symbolism, design of restaurant and supermarket, product packing, etc., and previous experience of the eating quality of the product. Personal factors involved in selection include age, ethnic group, class and mood. The chapter continues with the mechanics of vision including colour vision deficiency. The requirements for appearance analysis within the wider concepts of sensory analysis, e.g., panel selection and controlled lighting, are described.

In Chapter 3, on measuring the colour of food, D. B. MacDougall describes the physics of colour measurement and the development of colour spaces including the most used CIELAB uniform colour space. Examples of some of the procedures that can be used to measure different types of food with their many and various problems are given. These include meat, orange juice and coffee, typical products where the food's colour pigment interacts with the light-scattering components of its structure. The effects of structural variability that lead to the perception of discontinuity in breakfast cereals and the differences between measured colour and visually perceived colour are discussed. The use of the Kubelka Munk analysis to differentiate between absorption and scatter is illustrated. The variables in the optical properties of spectrophotometers and colorimeters that affect measured colour values are discussed, as are the effects of different lighting conditions on the perceived and measured colour of the food.

Stephen Westland continues the topic of human perception of colour in Chapter 4. He describes the retinal image and the effects of the surroundings on the perception and appearance of colour, especially the phenomena of colour constancy and colour contrast. The importance of white as a reference point in the field of human vision and its role in adaptation is presented. The development of colour appearance models is outlined and the relationship of these uniform colour space models to their use in the formation of digital image

#### 4 Colour in food

quality assessment is presented. Particular attention is drawn to the CIECAM97s model, which successfully relates the surroundings, the illuminant, colour contrast and brightness in the complicated transforms required to achieve cross-media colour reproduction.

Chapter 5 by P. Joshi presents a variety of examples of reflectance colorimetry in the measurement of food materials. The author presents a systematic approach to the problems facing anyone trying to measure food colour with the choice of many different types of instrument available. She describes the process of colorimetry, instrument standardisation and the problems of sample preparation for reflectance. Five different types of foods with their appropriate measurement techniques are described, opaque powders, granules and flakes; opaque particulate or lumpy materials; large area solid foods; pastes and slurries; translucent and transparent liquids. She concludes by pointing out that there is still a lack of information concerning many of the essential areas of measurement that are applicable to foods.

Chapter 6 by S. C. Bee and M. J. Honeywood tackles the industrial case of sorting food by its colour. High-speed instruments currently are used to separate faulty products, e.g., contaminants such as glass and extraneous vegetable matter, from the bulk of the material. The basis of the equipment is described under the headings of the feed system, the optics, the ejection process and the image-processing algorithms. Machines vary depending on the size and nature of particles to be handled. To determine the type of sorting machine required for a particular product, spectrophotometry of the normal and abnormal product is required before the appropriate wavelengths are selected for maximum separation. Monochromatic, bichromatic, dual monochromatic and trichromatic techniques are described as are the use of fluorescence, infra-red and laser techniques. Examples are given of typical foods along with their performance rates.

Chapter 7, by B. Moss is the first chapter in the second part of the book and deals with the subject of food colorant classification and colour chemistry. The distinction between dyes and pigments is defined and problems of natural, nature-identical and synthetic food colour are discussed. The chemistry of the various types of food colours is presented relative to their chemical classification. These are the isoprenoid derivatives, typified by the carotenoids; benzopyron derivatives where anthocyanidins are compared with the tannins; tetrapyrrole derivatives which contain a central metal atom surrounded by pyrrole rings containing the important pigments chlorophyll, hemoglobin and myoglobin; the melanins and caramels, products of nonenzymic browning. The relationship between chemical structure, light absorption and colour is reviewed and the reactivity of the colorants as affected by heat, irradiation, pH, oxidation, etc., is discussed.

Chapter 8 by U. Kidmore *et al.* deals with specific problems in the colour stability of vegetables. The occurrence and chemistry of vegetable pigments, e.g., chlorophylls, carotenoids and flavonoids and betalains is described and their colour stability and degradation detailed. The breakdown products of chlorophylls are listed and the severity of oxidation of carotenoids is discussed, etc. The post-harvest influences on vegetable colour and the factors involved in colour change, i.e., temperature, microbial growth and physical damage, are

related to effective shelf life. The importance of the effects of heating and freezing processes on the degradation mechanisms is discussed in detail. The overall objective in vegetable processing of maintaining maximum colour stability is emphasised in the chapter.

Chapter 9 by M. Jakobsen and G. Bertelson, on maximising meat product colour quality by modelling, reviews the many influences that affect meat colour stability. Maintaining the bright red oxymyoglobin colour in packaged meat can be affected by both external and internal factors. The external factors include headspace gas composition, package film permeability and gas absorption by the meat. Examples of modelling the external factors for fresh beef and cured ham are given. Internal factors for fresh meat are associated with meat type and metabolic rate and for cured meat nitrite level and vitamin activity are important variables. The success and usefulness of the models is presented with recognition of their limitations.

Chapter 10 by F. Artés *et al.* is concerned with the analysis of changes in fruit pigments. The type of pigments, their occurrence and location in the plant is discussed relative to their biosynthetic and degradative activity. The pigments are grouped according to their characteristics. Different biochemical pathways are involved in the changes in each class of pigment during the ripening of fruit and some of these are discussed in detail. Quick and simple analytical methods for measuring product quality are necessary for the food industry and applications of the use of colorimetry, spectrophotometry and chromatographic methods are described.

Chapter 11 by I. Amaya and V. Valpuesta presents the possibility of improving natural pigments in plants by genetic modification. Genetic engineering is a new approach developed during the past two decades. Understanding the roles of plant genes in the biosynthetic pathways of pigmentation is progressing, although not as rapidly as the more important research into pathogen defence and herbicide resistance. The authors describe the various pigments in fruit and flowers. The incorporation of genes from one plant to another for colour improvement has been shown to be possible in experiments with flowers where new colours have resulted where plants have been transformed by incorporation with a foreign gene. In transgenic studies on tomatoes, using bacterial genes, the carotene content has undergone a fourfold increase. Indications are that the potential for further improvement in plant colour is highly possible.

Chapter 12 by F. J. Francis reviews current developments in colourings used to alter or enhance colours naturally present in food. It covers the chemistry, usage and safety of the range of synthetic colourings. It also considers the growing number of natural colourings, such as carotenoids, which are increasingly replacing synthetic colourings as a result of consumer concerns about the safety of the latter.

Chapter 13 by S. R. Nielsen and S. Holst on development in natural colourings builds on Chapter 12 and focuses on the application of natural colours in food and beverage products. The most used natural colours range from yellow through orange, red and green to brown and black. The entire range is of plant origin with the exception of cochineal. Caramel and carbo-vegetabilis,

carbon black, are also manufactured from plant materials. The general principles for the application of colours to products are described with the overall aim of matching what the consumer expects from the particular food product. Selection of appropriate methods of application, e.g., whether the colours are water-soluble or oil-soluble, is vitally important. Account must be taken of the effects of pH and processing, especially browning from the Maillard reaction and loss of initial colour. The chapter presents the necessity for good storage practice of the colours and the necessity for establishment of quality control procedures.

The final chapter in the book (Chapter 14) by J. B. Hutchings *et al.* assesses the practice and future use of using image analysis (IA) for food appearance measurement. IA techniques have the advantage that, in addition to measuring colour, they can be used to assess other important appearance aspects of the overall appearance quality of foods. The colorimetrically based characterisation of VI camera output and the mathematics of the recovery of spectral information are outlined. The DigiEye system of obtaining camera images in defined illumination conditions is described. Its application to food is shown to be effective in the examples of the image analysis of bananas and breakfast cereals. The chapter finishes with the potential of relating VI information to the sensory analysis of foods. Although the food industry's needs vary the uptake of VI techniques by the industry would seem to be assured.

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## **Part I**

### **Perceiving and measuring colour**



## 2

# The perception and sensory assessment of colour

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### 2.1 Introduction

It is a common misconception among food scientists and technologists that the colour of the product is the major visually perceived factor influencing customer selection. Choice is, in fact, governed by a hierarchy of appearance properties of which the colour is just one. This hierarchy, which results in the formation of expectations, is controlled by the order of importance of the constituent elements of appearance. Colour is the paramount attribute influencing the selection of paints while colours and shapes are the paramount attributes influencing selection of clothes but it is total appearance and expectations that govern food selection. The information transfer process describes the derivation of images arising from the total appearance of the scene, that is, from the properties of the product or scene and properties of the viewer looking at the scene (Hutchings 1999).

### 2.2 Expectations and the information transfer process

The sight of food, that is, its total appearance generates expectations (Hutchings 2001). These are, in turn, are influenced by the event as well as by the viewer's needs and wants at that particular time. Expectations comprise:

- visual identification – what is it?
- visually assessed safety – will it harm me?
- visually assessed flavour – what will its flavour be when I consume it?
- visually assessed texture – what will its texture be when I consume it?
- visually assessed satisfaction – is this food item appropriate and sufficient for my present needs, will I be satisfied?



Hence, the total appearance of a particular food communicates in a number of ways. First, it tells us about the food itself and second, through a halo effect, about the properties of other foods.

First, total appearance of the food tells us about the food itself. The colour, size, shape and surface texture tell us that this object is an orange. Through what we have learned about oranges, these properties also may tell us that it is ripe, mature, perhaps juicy, not rotten in the middle and is good enough to eat. Also, the appearance tells us that this orange is healthy, that it is full of those antioxidants and free radical scavengers that play vital roles within our cardiovascular system. These are positive expectations.

There are negative expectations also. The colour uniformity resulting from the blemish-free surface of this orange tells us it may have been heavily treated with pesticides and herbicides, and therefore that the fruit as well as our hands must be washed before we eat it. Also from the glossiness of the skin we must expect that a coating of wax has been applied after harvesting to prevent loss of moisture, and therefore that we must scrub it before using the zest for cooking. So total appearance enables us to recognise a food as well as helping us to recognise its quality and set up our expectations prior to eating it.

Second, the total appearance, hence expectations, of the orange itself tells us about the properties of other similarly coloured foods. Fresh orange juice may have the same health-giving properties as the orange itself, but orange-coloured products containing added water, sugar and only 5% orange juice do not. Hence the information transfer process, through which we derive images and expectations, is complex but it provides methods for studying and understanding the formation of appearance images and expectations relevant to a wide range of situations. These include the product, the package, the pack in the freezer and shop, and the design of the restaurant and supermarket; in fact, to all appearance images however they arise.

Within the information transfer process, designers, developers and manufacturers manipulate material properties to create a scene consisting of scene physics. The scene encourages in the viewer basic perceptions of the scene as well as total appearance images and expectations of the scene. We can use Appearance Profile Analysis to assess formally images and expectations of the scene, and we can thus attempt linking scene material properties with total appearance images. The scene in view can be described in terms of the physics of the elements of the scene coupled with the physics of the way the elements have been assembled, that is, the design. The scene physics and design working together contribute to the stimulus which is converted into appearance images in the brain of the perceiver of the scene. There are two broad types of image, basic perceptions, such as size and colour, and derived perceptions (or, visual expectations), such as creaminess and value.

A product or scene possesses physical properties that can be summarised as spatial (properties of dimension), spectral (properties dependent on wavelength of light reflected or transmitted), goniophotometric (properties dependent on angles of illumination and viewing), and temporal (properties dependent on

movement and time). The product viewed under illumination, which itself can be defined, results in two types of perception. Basic perceptions are of size, shape, surface texture, colour, translucency, gloss and their patterns and uniformities. Derived perceptions, formed through repeated eating experience, comprise visual expectation (described above). The extent to which these expectations are subsequently confirmed or otherwise has a profound effect on acceptance.

When viewing a scene, our images are normally *Gestalt* expectations or derived perceptions. Nevertheless, they are linked with the basic perceptions through the specific properties of the viewer's visual mechanisms. The total appearance model includes consideration of appearance images, what they are, how they arise, how they can be measured, and how they can be manipulated. It can be applied to any situation in which individuals find themselves, but applications described here are confined to foods.

## 2.3 Total appearance

The total appearance of a scene comprises the visual images within us. These images are controlled by viewer-dependent variables and scene-dependent variables. Viewer-dependent variables consist of the viewer's individual visual characteristics, upbringing and preferences, and immediate environment. Scene-dependent variables consist of the physics of the constituent materials and their temporal properties combined with the way these are put together, and the scene illumination providing light and shade to define the volume and texture of the scene. The model considers the build up of appearance images.

### 2.3.1 Product images

Appearance images are dynamic and can be broadly classified under three headings. These are the on-the-shelf images contributing to the buy decision, the in-preparation images contributing to the decision to continue preparing the product, and the on-the-plate images which determine whether or not we eat it. Throughout this process experiences are compared with expectations. Such images form a significant part of any repeat-buy decision. The on-the-plate image consists of a mixture of individual appearance attributes which must fulfil physical and emotional expectations. What is on the plate should be stimulating; should look sufficient to cope with the appetite, large or small, of the moment and should live up to expectations, whether derived from advertising claims, prior experience or personal mental image of the product. Within each of these product situations, in addition to expectations, it is postulated that there are two basic types of appearance image, 'immediate' and 'considered'.

The immediate image is the *Gestalt* or impact image comprising the initial recognition of the object or scene plus an initial judgement of quality, for example, 'I like the look of this meal'. Considered images are of a more

thoughtful kind comprising sensory, emotional and intellectual images. These descriptors are not rigidly categoric but are useful, together with expectation types described above, in prompting questions to be asked of a particular scene. In an eating situation, a sensory image includes an assessment of visually perceived sensory properties, for example, ‘This yellow dessert will taste of lemon’. It is a response mainly to the food and the décor, but not to the event (that is, the reason for the meal). An emotional image may have positive or negative connotations and includes the reasons for the meal, for example, ‘I always eat chocolate coloured blancmange to celebrate my birthday’, or ‘What a waste of money!’, that is, emotion by physical association or recall. An intellectual image might raise questions of the food, décor, the company or the occasion, for example, ‘I wonder how this was made?’ Responses to these images range from eating with relish to an abandonment of the meal.

From time to time issues arise affecting the relationship between the customer and the retailer. These include concerns about the environment, animal welfare, energy or pesticide usage, value for money, food labelling, customer health, genetic modification of foods, and fat and sugar contents. Any of these aspects of appearance can dominate the *Gestalt* judgement of degree of acceptability.

## 2.4 Viewer-dependent variables

Viewer-dependent variables consist of the viewer’s:

- receptor mechanisms
- inherited and learned responses to specific events and situations
- immediate environment.

These variables are amplified in Table 2.1

### 2.4.1 Receptor mechanisms

Illumination and object properties interact to provide the stimulus for the receptor mechanisms. There are four parallel systems concerned with different attributes of vision – one for motion, one for colour and two for form (Zeki 1992). Perceptions of colour and appearance are unique to the individual as receptor response is governed by the inherited and acquired visual characteristics of the viewer. They change with colour vision ability, state of visual adaptation, after image, colour constancy, discrimination and metamerism characteristics. These properties carry the effect of viewer age, but other aspects of ageing affect response. These include cataract, response to glare, intensity need, and yellowing of the macular pigment. Information received about the object from the other senses, hearing, smell, taste and touch may also affect total appearance judgements. The weight given to each individual sense when making judgements is different for different people. Although visual, olfactory and taste

**Table 2.1** Total appearance – viewer-dependent variables (Hutchings 1999)

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**Receptor mechanisms**

Inherited and acquired sensory characteristics consisting of

- colour vision: adaptation, after-image, constancy, discrimination, metamerism
- ageing effects: cataract, glare, light intensity need, yellowing
- other senses: hearing, smell, taste, touch.

Inherent and learned responses to specific events and situations

- culture
- memory
- preference
- fashion
- physiological effects
- psychological effects.

Immediate environment

- geographical factors: climate, landscape, seasonal change
  - social factors: crowding, personal space, degree of awareness
  - medical factors: survival and need, state of well-being, protection.
- 

sensitivities decline with age, older members of the population are more sensitive to colour cues and less sensitive to changes in flavour concentration (Cowart 1989). They also perceive colours as having less chromatic content than those who are younger (Pumpian-Mindlin 1954). Visual receptor mechanisms are discussed further below.

#### **2.4.2 Inherited and learned responses**

Information from receptor mechanisms is processed and judgements made are modified according to the perceiver's specific upbringing and experience. This includes inherited and learned responses to specific subjects and activities. Governing these are such considerations as culture, tradition, fashion, memory, preference and prejudice.

All cuisines are governed by a communally determined and accepted set of rules. Eating itself acts as a social bond as eating patterns are part of group behaviour. Hence, some foods may be seen to have a higher social status than others. Food is also a form of communication, as generally we do not give friends food items because we think they are hungry.

Food selections are strong markers of age, ethnic group and class. For example, young children prefer strong bright colours, and this extends to their choices of sweets and desserts. By adolescence, colour preference has changed to pastel shades (Lyman 1989). The 'child' view of foods is of sensory reinforcement and fun, while the 'adult' view tends strongly toward health and other adult-orientated messages (Moskowitz 1983). Factors affecting purchase

choice by adults are our perceptions of taste, nutrition, variety in preparation, compatibility with other foods, usefulness as snacks, availability, cost, attainability within the food budget, level of expectation, and quickness and ease of preparation. At the eating stage, foods may be disliked because of quality, size of serving, food temperature, monotony, over familiarity, menu cycles, and standardisation of preparation and presentation (Kahn 1981).

Colour preference for clothing and decor is heavily dictated by fashion, and hence will be relevant to colours used in the food environment. However, for food itself this is not so. Food colour is vital to our well-being, our visual characteristics probably evolved to ensure the successful selection of good-quality food. Hence we can redecorate the store in different colours from time to time, but we cannot change the colour of staple food items without customer rejection.

There are four types of colour association or symbolism: associational, acculturated, symbolism of the familiar, and archetypal symbolism. These are relevant to many areas of life including foods. Associational symbolism concerns personal experience that arises from upbringing and education. Colour forms a major part of associational symbolism in the marketplace. White is associated with dairy products, softer brown and golden tints with expensive luxury foods. In some stores, colour is used to identify specific locations. On a cold day we feel warm at the sight of a hot meal, but the same material can change in association according to current environmental conditions. The sight of a meal changes from being welcome to being not needed when an unwelcome visitor arrives. Acculturated symbolism concerns cultural influences. For example, roast beef and Yorkshire pudding are the essence of Englishness. Food itself is symbolic and can be accepted as such without regard to its nutritional value. Some peoples specify rigid food acceptances or taboos, the latter leading to starvation in the presence of healthy food.

Symbolism of the familiar concerns the routine of everyday life. Everyday foods of correct appearance eaten at normal mealtimes represent security. An incorrectly or unnaturally coloured food arouses suspicion and is unsettling. Archetypal symbolism is the symbolism of psychologists. It is that which may lie in the subconscious part of our personality, for example, red and yellow colours are called *warm*, blue and green *cold*.

### **2.4.3 Immediate environmental factors**

Immediate factors of the environment affecting total appearance include geography, season, climate and landscape character. Our physical and social situation comprises crowding, our personal space, the company we are in, how much money we have, our general medical state, our appetite. While shopping, we are aware of the way foods are packaged and arranged in displays, the choices available and what others are buying. We are aware of store colours, cleanliness and how easy it is to get around. Each perception involves memories, concepts and attitudes and these in turn affect, and indeed are affected by, the

way we feel at the time. The cognitive set of an individual affects attitudes to particular foods. Many such features in the environment, independent of the food we are eating, influence our opinion of the food and success of the occasion. These are ‘halo effects’ and are discussed further in the section on sensory assessment.

Whatever the eating situation a variety of sizes, shapes, surface textures and colours are required to tempt us to eat. Both serious and casual eaters prefer food images to be positive. Illness has psychological repercussions affecting attitudes, often taking the form of pickiness and finickiness. Older people tend to be more health conscious (Smails 1996). On the other hand, meals for the sick should be attractive, appealing and appetising, even to the extent of sacrificing nutrition (Pumpian-Mindlin 1954).

## 2.5 Scene-dependent variables

Scene-dependent variables are shown in Table 2.2. They consist of the design tools, that is, scene materials and lighting, and the design itself.

### 2.5.1 The design tools

Materials and lighting are design tools. There are three types of physically definable material properties – optical properties, physical form, and temporal properties. Optical properties include light distribution occurring over the surface and within the depth of the material as well as reflectance, transmission,

**Table 2.2** Total Appearance – scene-dependent variables (Hutchings 1999)

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Material physics of the scene and scene elements

- Material properties
  - optical properties: spectral, reflectance, transmission, goniophotometric
  - physical form: shape, size, surface texture
  - temporal aspects: movement, gesture, rhythm.
- Lighting of the scene
  - illumination type: primary, secondary, tertiary
  - spectral and intensity properties
  - directions and distributions
  - colour-rendering properties.

The designer designs for

- communication for identification, safety, symbolism
  - aesthetic reasons in different forms, from one-dimensional writing, through two-dimensional pictures, to three-dimensional architecture, to four-dimensional performing arts.
-

spectral and goniophotometric factors. These properties can be used in specifications of visually perceived object colour, translucency, transmission and gloss. Physical form includes statements of size, shape, pattern, surface texture, and those strength or viscosity properties that become visible by virtue of the temporal aspects of the object. Temporal properties include attributes that change with time, such as ageing and the way a material deforms under pressure or the jelly that wobbles when shaken.

Design involves the creation of a whole from materials building blocks and lighting. It has two functions, to make the product or scene communicate, indicate or symbolise, and to make the product or scene look good. Colour can be a primary part of an object, for example, the red of the traffic light. Colour can also be a secondary property, such as that incorporated in a barely noticed room decoration. However, for most foods the colour is a primary property, it tells us about the eating quality.

As a primary property food colour identifies, it advertises that a fruit is ripe and directs us to harvest, or it informs us the roast meat is ready to eat, it arouses our expectations and anticipation, and motivates us to eat. If the meat is green, the colour commands us not to eat. Colour of fruit and vegetables is a symbol that reinforces our belief that we are eating a healthy meal that will perhaps initiate a cure for our current condition. We eat a piece of traditional white and blue wedding cake so that it will bring good luck to the happy couple.

Colour and design are used for aesthetic reasons, that is, they contribute to conspicuity, and they decorate, please or placate. It can be helpful to consider aesthetic judgements in terms of performance or dimensional complexity. Of a single dimension is the written word; two dimensions a painting, three, a package, a live landscape or an architectural scene, and four, an active artistic performance. Food products by themselves are mainly three-dimensional, dimensions one and two being added by the package, and four revealed during food service, perhaps in the restaurant.

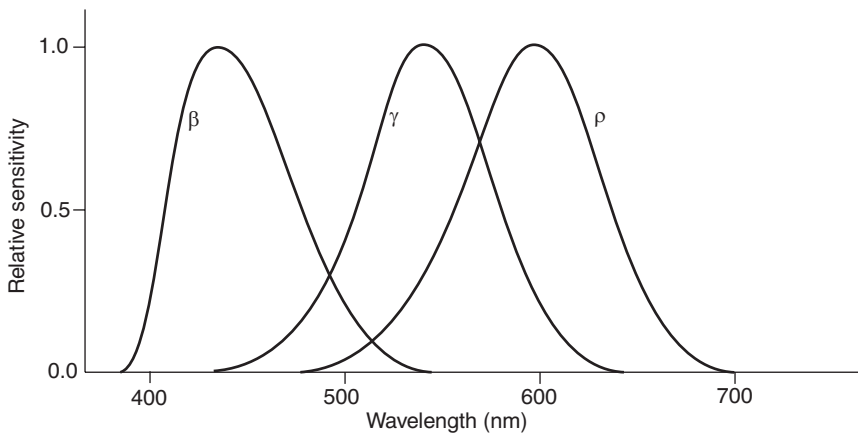
### **2.5.2 The lighting**

Light and shade define the volumes and textures of the scene in view. Lighting properties, such as spectral intensity, directional distribution, and colour rendering ability, also affect the perceived colour, gloss and translucency of objects within the scene. The eye can become accommodated to tungsten lamps and all fluorescent lighting designed for the home. That is, white or near white objects will appear white. Although butchers normally take care to present their raw beef under red-biased illumination, some meat cabinets are illuminated with very red light. This defeats the purpose of the exercise, as the eye cannot become accommodated, whites become pink, and the meat can appear unnaturally red and almost fluorescent. Lighting can be used to great effect in the food industry although no lamp is yet available that can attractively present all foods. Lighting is further considered under sensory assessment.

## 2.6 The mechanics of vision

The retina acts as a transducer between light entering the eye and the processes of light and colour perception taking place in the visual cortex of the brain. The light-detecting elements of the retina are the morphologically distinct 120 million rods and 7 million cones. Approximately 1 million ganglion cells carry information from the retina to the optic nerve. At the fovea, the convergence is 1:1 while at the periphery it is several hundred to one. This degree of convergence determines spatial resolution and sensitivity. Hence, at the foveola, a spot 2 mm in diameter in the centre of the fovea containing only cones, there is low light sensitivity but maximum resolution to 1 minute of arc. This area is coloured yellow probably as a protection against ultraviolet light. The number of rods increases to a maximum approximately  $20^\circ$  from the fovea. Here, there is high light sensitivity and low spatial resolution. This is the angle used for improved sight in the dark, when we use averted vision. Outside this area colour discrimination is zero and the remainder of the retina is probably used solely for the detection of movement.

There are three types of cone each having a characteristic distribution of wavelengths over which it responds to incoming radiation. Under low levels of illumination rods function, cones do not; hence there is no colour discrimination. Cones operate in conditions of photopic vision, that is, at higher levels of illumination. At mid-illumination intensities there is a gradual shift from one type of response to the other. The spectral sensitivity of each type of cone is shown in Fig. 2.1. These curves are obtained using colour matching experiments, but spectral sensitivities can be confirmed from direct observations on individual cones using a microspectrophotometer. The three cone types have peak sensitivities in the blue, green and yellow-green parts of the spectrum.



**Fig. 2.1** Spectral sensitivity curves of the three types of cone that compose photopic vision (Hunt 1998). Reproduced from R. W. Hunt, *Measuring colour*, 3rd edition, 1998. ISBN: 0863433871. Published by Fountain Press, an imprint of Newpro UK Ltd, Old Sawmills Road, Faringdon, Oxon. SN7 7DS.



They are called respectively, the blue ( $\beta$ ), green ( $\gamma$ ), and red ( $\rho$ ) receptors. The different sensitivities to wavelength provide the foundation for colour vision. Light of different wavelengths impinging on the retina induce responses in the  $\beta$ ,  $\gamma$ , and  $\rho$  cones of magnitudes dictated by the spectral sensitivities.

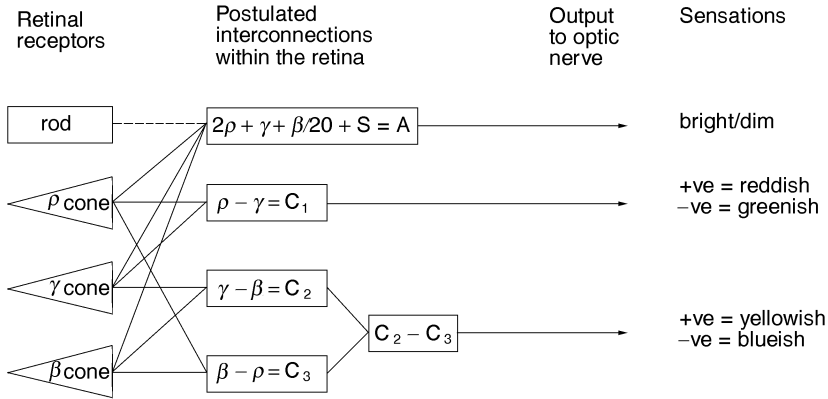
There are six specific areas, V1 to V6, within the visual cortex responsible for the perception of different aspects of appearance. V1 responds to orientation, real and imaginary boundaries, and has some wavelength response. It also detects overlapping features in the scene. Cells in V2 are sensitive to colour, motion, orientation and stereoscopic features. V3 is sensitive to form and depth. V4 is sensitive to colour and is the site responsible for the maintenance of colour constancy. V5 analyses motion and V6 is responsible for analysing the absolute position of an object in space (Lee 1997). Information from the retina is processed in two broad systems; one is concerned with identification of the object, the other with relative spatial position (Tov   1996).

Visual recognition occurs when a retinal image of an object matches a representation stored in the memory. The ability to recognise objects depends on visual neurons being able to form networks which can transform a pattern of points of different luminous intensity into a three-dimensional representation. This enables the object to be recognised from any viewing angle. Neuron cells responsible for specific recognition tasks have a columnar organisation. Adjacent cells usually respond to very similar feature configurations. These simple shapes form a 'visual alphabet' from which a representation of more complex shapes can be constructed. Some neurons, called face cells, may form a neural substrate for face processing. Such cells respond only to faces, whether in life, as plastic models or on the screen.

Moving objects can be tracked using pursuit eye movement. Considerable neuron interconnections are required to follow objects that are continuously displaced from one point to another. Two other types of eye movement occur. Continual small movements are needed to destabilise the image and prevent the retina adapting to a continuous stimulus, and larger short movements permit the eye to scan the visual environment. Three distances appear to be relevant within this depth perception. These concern the personal space occupied by our body, peripersonal space within reach, and extrapersonal space beyond (Tov   1996).

## 2.7 Colour perception

There is a highly complex series of connections between the detector and fibres making up the optic nerve taking the resulting signals to the visual cortex. This link can be depicted using a simplified hypothetical framework (Hunt 1998). There appear to be not four but three types of nerve fibre. One carries achromatic or brightness information, the other two carry colour information – the opponent colour mechanisms. This model is shown in Fig. 2.2.



**Fig. 2.2** A postulated representation of the interconnections between receptors and the subsequent signals on which colour sensations depend (Hunt 1998). Reproduced from R. W. Hunt, *Measuring colour*, 3rd edition, 1998. ISBN: 0863433871. Published by Fountain Press, an imprint of Newpro UK Ltd, Old Sawmills Road, Faringdon, Oxon. SN7 7DS.

Colour information from the retina is obtained from a series of signal differences.

$$\rho - \gamma = C_1$$

$$\gamma - \beta = C_2$$

$$\beta - \rho = C_3$$

These are transmitted as two signals,  $C_1$  and  $(C_2 - C_3)$ . The achromatic signal under daylight conditions comprises all three  $\rho$ ,  $\gamma$ , and  $\beta$  responses, weighted to compensate for the differing number of each type of cone (40:20:1) in the retina. That is,

$$2\rho + \gamma + \beta/20$$

Hence, the total achromatic signal can be described as:

$$A = 2\rho + \gamma + \beta/20 + S$$

where  $S$  defines the rod response. When  $\rho = \gamma = \beta$ ,  $C_1 = C_2 = C_3 = 0$ . The value of  $A$  indicates the level of brightness and greyness (degree of whiteness or blackness).

## 2.8 Colour vision deficiency

Approximately one man in 13 and one woman in 250 perceive colours in a markedly different way from the remainder of the population. The other 92% of the population do not perceive colours in exactly the same way, the cone

response curve shapes varying between individuals. Colour vision deficiency in relation to viewing foods has not been well researched, but if visual impressions are important, colour defective individuals may substitute other parameters for colour in their food appreciation. Individuals with poor colour perception learn the socially approved colour names for many objects (Amerine *et al.* 1965).

Colour deficient vision can be inherited or acquired as a result of retinal or optic nerve pathology. Deficiency can arise from illness or ingestion of neurotoxic agents, although the most common acquired form is from cataract and glaucoma in old age. As we grow older our colour perception and sensitivity decline. Subjects in their 60s and 70s perceive coloured surfaces as having less chromatic content than those under 30. This is not caused by physical changes within the eye but possibly caused by changes in neural mechanisms responsible for processing hue (Scheffrin and Werner 1993).

Defects arise when the response functions of the cones, or very occasionally the rods, are different in some way. The most common causes relate to a  $\gamma$  cone deficiency, 5% (deuteranomaly), or an absence, 1% (deuteranopia); and the deficiency, 1% (protanomaly), or absence, 1% (protanopia), of the  $\rho$  cones. Defects or absence of the  $\beta$  cones is unusual. The presence of these conditions can be detected by using Ishihara charts which are based on colour confusion. They consist of a series of plates made up of spots of different colour. The spots may wrongly be described as being the same colour, and hence the charts are known as pseudo-isochromatic tests. These can be used to detect the presence of a colour vision deficiency, but not necessarily give information as to the type. Colour deficiency is a significant handicap only in those technical situations requiring the individual to perform exacting colour tasks, such as mixing colours to produce a generally acceptable colour match. These skills are required, for example, in the textile dyeing, ceramics and paint industries. Problems in the food industry have included tomatoes being picked while they were still unripe, and green sweets being produced instead of red ones.

A list of jobs, careers and industries that have different degrees of vulnerability to abnormal colour vision has been compiled by Fletcher and Voke (1985). In the food and agriculture industries those who select and grade raw materials and products on the basis of colour ought to be chosen with care. For a potential grader in the industry, a screening with the Ishihara charts, although not perfect, will probably be sufficient to detect unsuitable candidates. These charts must be used according to the strict instructions supplied and under the recommended lighting and viewing conditions. Further investigations using more complex colour vision testing techniques must be carried out by someone with training and experience in their application and interpretation.

## 2.9 Sensory assessment of appearance properties

Our internal dictionaries of appearances are built up from the permanent memory, itself built up from direct stimulation from the sensory receptors, and

the short-term working memory. These enable us to recognise good and bad quality. Such judgements are individual and many perceptions contribute to appearance in the wider sense. Among these are knowledge, belief, convenience, price, prestige, familiarity, risk assessment, naturalness, taste, tolerance and satiety (Kronidl and Lau 1982). Unless our quality judgements have been deliberately trained, say for participation in an expert panel, they will be purely the result of our own personal past experiences and prejudices.

Within the food industry, vision is the most commonly used means of assessing attributes of appearance. In the paint industry, however, colour measurement is firmly established because colour can be isolated as a single visual and instrumental property. Full confidence can be placed in the results obtained even for the high precision and accuracy needed for matching paints. On the other hand, the complex make-up of the appearance properties of foods, their heterogeneous nature, and the short season or batch nature of manufacture, may make traditional sophisticated colour measurement and matching techniques unnecessary, unworkable and uneconomic. Problems arising from agreeing and defining individual appearance attributes, and from the labile nature of foods can normally be solved by discussion. However, new digitally based technology will greatly improve our ability to make routine colour and appearance measurements – see Chapter 14. Appearance control is necessary also because mass marketing methods have trained customers to expect a high degree of product uniformity. This places additional constraints on the producer. When assessors agree, suitable scales can be devised and samples scored in a disciplined way.

There are some manufactured products for which consumers seem to have a special sensitivity to colour. Examples of these are branded tomato ketchup and milked brewed tea. In such cases it is essential that raw material selection, categorising, and blending techniques are rigorously established, and manufacturing and marketing techniques carefully defined and controlled. A common factor across all industries, to which colour and appearance are important, is that formal sensory assessments of appearance must be carefully planned, controlled and executed. The objectives of the assessment and the purpose of the work must be understood and agreed. The running of sensory panels can be expensive in time and labour, therefore the questions asked of the project and the techniques employed by those in control of the testing must be relevant to agreed objectives. Adequate selection and training of panellists is essential for the effective and efficient performance of the panel.

## **2.10 Panel selection, screening and training**

Selection and training of appropriately qualified and motivated persons for participation in analytical panels is essential to effective performance. Individuals participating in panels in which colour judgements are involved should have been screened for colour vision deficiencies using Ishihara charts. However, other tests may be necessary for the optimal performance of certain

visual tasks. The 100 hue test is useful in testing colour perception skills; the Bodmann test, used to test speed of response, is a search task consisting of numbers randomly scattered over a sheet of paper. Subjects are asked to find the positions of specific numbers and the time taken is an indication of their search speed. The Land Halt Ring and Maze Tests involve the subject in finding gates in circles or mazes. These are recommended for screening those who, for example, sort fruit on high-speed grading lines (Fletcher 1980). A battery of tests has been assembled for defining inspection performance in the engineering industry. These include acuity, inspection, sorting and memory tests (Gallwey 1982).

Much has been published concerning the training of members of sensory panels; particularly those concerned with quantifying flavour, taste and aroma. Curiously, very little has been published on the training of panels for quantification of visual attributes. Sensitivity to food colour differences can be tested using materials appropriate to the project. For example, where the quality of a particular product depends on browning, each panellist can be asked to rank solutions of caramel and heat-treated milk as a test for sensitivity (Jellinek 1985). Similarly, sensitivity to other appearance factors, such as translucency and gloss may be determined using model samples and by discussion and trial. This involves using a series of samples, preferably food, possessing a range of the attributes in question.

Distribution and uniformity of colour, translucency and gloss can be quantified according to the specific problem. Proportions of a discontinuity within an area of colour (for example, proportion of green on the surface of a tomato) may be expressed as a percentage of the surface area, or by using a simple rating scale. Each product application has specific requirements involving the possible development of suitable grade definitions, scales and anchor points (Hutchings 1999). There are many areas of the industry in which expert experienced judgements are required. These grading procedures are satisfactory and can work well, but discussion, agreement of attributes comprising the grade, adequate training, and retraining are vital to their success and uniform application within the particular company and industry.

## **2.11 Factors affecting panel performance**

Many personal and environmental factors upset panellist performance. Care must be taken in panel design to counterbalance or eliminate all possible extraneous variables, including practice and fatigue (O'Mahony 1979). Individuals behave differently but normalising scores can reduce score variability. This can be achieved using the hypothesis that subjects behave in scaling as instruments working with different sensitivities of range and scale of response and different zero setting. The score of each panellist is recalculated by normalising sensitivity of each subject to the averaged sensitivity of the panel (Myers 1990).

Panellists not performing as normal can sometimes be eliminated. For example, wine judge agreement, reliability, discrimination and stability varies

with time. Monitoring of judges reveals this and their results can be omitted from analysis (Brien *et al.* 1987). How we feel at the time of the assessment may affect the judgements made. In a series of acceptance tests, prior interview established two groups of panellists, those feeling positive and those feeling negative about their current physical state. Acceptance ratings of dairy bars were significantly higher for the positive group (Siegel and Risvik 1987). Hunger and monetary reward also affect performance. Rewarded subjects and hungry subjects rated samples of breaded fish higher than subjects who were not rewarded or pre-fed (Bell 1993).

Orientation of the panellist towards the tasks by trial tasting prior to taste difference testing can improve performance in triangle tests (O'Mahony *et al.* 1988). Such an effect might be demonstrated for visual tasks. Although many individuals enjoy interacting with computers, fears have been expressed over possible increases in anxiety and feelings of dehumanisation. However easy the panellist's task, cost in financial and human terms may become a problem (Armstrong *et al.* 1997).

As discussed in the section on inherited and learned differences between individuals, it is almost inevitable that preference can often be linked to race, environment and background (Hutchings *et al.* 1996). Changing lifestyles affect food choice. For example, there are two groups of people illustrating the influence of social trends on health perception, those whose attitudes can be termed 'return to nature' and those with 'acceptance of disorder' feelings. The majority in the former group believe special measures are necessary for health care, are concerned about eating sugar, carbohydrates, animal fat, white bread and salt, that too many artificial ingredients are added to food, and that only packs which state nutritional values should be purchased (Lowe 1979). Such preconceptions affect panel scores (Lundgren 1981) but with adequate training this tends not to occur with expert panellists (Deliza *et al.* 1996). Different ethnic backgrounds lead to the use of inappropriate scales in panelling. For example, those used successfully in Europe and North America cannot be transferred directly to an urban African population without pretesting. Unfamiliar concepts may include equal interval and continuous rating scales, graphic and visual scales, and the giving of marks in numerical scales (Morris and van der Reis 1980).

Whatever the requirements and questions, those in charge of a panel need to make certain that panel members are thoroughly conversant and practised with the definitions, descriptions and scales to be used before the assessments proper start. Those in charge must also be aware, as far as possible, of those factors affecting individuals and groups, and which may influence direction and magnitude of scores.

Colour and appearance are powerful indicators of object quality. This applies particularly to food. Human beings have different sensitivities to flavour and it is relatively easy to confuse tasters by giving them inappropriately coloured foods (Moir 1936). The existence of the halo effect results in some foods having to be tailored for a particular market. For example, the French prefer their rosé wines to

be an onion skin colour, the British prefer a light pink, and the Germans and Swiss a deeper pink. There is a division within the United Kingdom for preference of tomato soup colour. One group prefers orange-red, the other a blood or cherry-red colour. Members of the former group were more likely to have been brought up on Heinz Cream of Tomato soup, which contains orange added beta-carotene, the latter were more used to eating purée or powder-based soups.

## 2.12 Halo effects

Halo effects arise from a number of sources during sensory testing (Hutchings 1999). Sample appearance, the environment and panel organiser attitude influence panel scores of in-mouth attributes. Panellists may be influenced by the way in which instructions are given, clues may be given to the answers expected. A halo from the physical environment arises when branded food packages or other foods are seen in the vicinity of the test area. Influences arise from the wider environment, perhaps a plush hotel or a run-down community hall. In the panel situation, not everyone is affected by the look of the product being tasted. There are two groups of subjects, field-independent and the field-dependent. Members of the field-independent group attend to their taste and smell perceptions when classifying flavour, without regard to what may be an inconsistent visual stimulus. The field-dependent subjects made more mistakes when trying to identify flavours in the absence of visual cues to their origin (Moskowitz 1983).

Sometimes, sensory attributes are judged at the same time and under the same conditions. Scores may be given for the flavour and texture attributes of a product that also varies in colour. Sometimes a high correlation between attributes is reported without comment of the fact that panellists may be influenced by appearance. For example, harvest time affects the sensory qualities of pecans. A later harvest yields darker, tougher kernels possessing greater off-flavour. These nuts have a decreased preference for colour, appearance, texture, flavour, a lower overall acceptability, and a consequent lower intention to buy. Pecans are, therefore, a good example of a food material for which it will be essential to eliminate all visual cues when independent detailed studies of flavour and texture are required (Resurreccion and Heaton 1987). Similarly, hedonic scores for red apples having the flavour of green apples increased when the flavour of the peeled apples was assessed (Dailliant-Spinnler *et al.* 1996).

Size constancy effects occur during multi-mode examination (Cardello and Segars 1989). The non-existence of perceptual size constancy can affect product appearance testing. For example, the British have been subjected to the persistent advertising claim that 'smaller peas are sweeter'. An unwary approach to the panelling of peas can result in erroneous findings founded upon knowledge of this claim.

Brand loyalty plays a significant part in panel results for many products including coffee (Moskowitz 1985), and beer (Allison and Uhl 1964). Brand-

loyal subjects have an enhanced perception of product properties for that brand. When the branding is obvious, the belief raises expectations and consequently higher scores are obtained (Sheen and Drayton 1988). Sensory panelling may concentrate too much on the product alone or on product concepts, and the lifestyle and attitudes of potential customers. Little account is taken of the extreme influence of brand (Martin 1990).

Colour influences other sensory characteristics and hence food discrimination through learned associations. The major mechanism prevailing within colour/taste and colour/aroma interactions is one of association with a specific product and specific product type. Wine and beer flavours are greatly affected by colour and assessments of port wine aroma and flavour are influenced by the ability to see the samples (Williams *et al.* 1984). Provided the flavour does not depart too much from that expected from its colour, the colour appears to determine the quality of red wine (Timberlake 1982). Characteristic colour/taste/flavour associations of specific fruits arose with evolution of colour vision skills. We appear to have a learned response to colour and sweetness through orange or red fruit being normally riper, more edible and sweeter. Similar associations for other flavours such as saltiness and bitterness are more doubtful. (Clydesdale 1993). Colour can also modify texture perception. For example, butter of a stronger yellow colour is perceived as being easier to spread. The educated consumer may be aware of effects of natural variations in fat composition with feeding and season of production and their relationship with colour and texture (Rohm *et al.* 1997).

### **2.13 Physical requirements for food appearance assessment**

Detailed guidelines issued by ASTM for the layout of general sensory evaluation laboratories are available (Eggert and Zook 1986). Design features particularly important for appearance assessment include the recommendation 'all evaluation areas, such as panel booths and training/testing rooms, should provide a comfortable, neutral, non-distracting environment'. Kitchen and preparation areas are sometimes coloured, but this is not advisable. There should be no other materials present in the examination area when samples of one colour are being evaluated and there should be no distracting or brightly coloured objects in the vicinity. A smock of neutral grey colour should cover bright or highly coloured clothing.

Computer terminals are installed in many individual booth areas. The ASTM recommended booth bench size of 30 inches (76cm) wide, and 15 inches deep, may not be sufficient to house the terminal as well as the sample. The terminal may also introduce distracting colour and contrast into the booth via the finish and screen colours. Any contrast introduced may be particularly intrusive during translucency assessments, or colour assessments of translucent samples. Therefore, the colour of the terminal and its organisation into the booth should be considered with care. Translucency becomes evident either when



illumination is from the back or from the front of the sample. In both cases introduction of contrast into the viewing area should be deliberate rather than accidental. In this way the viewing situation is under control.

It is necessary to control and standardise sample-handling procedures. Acceptance appraisals should be conducted under conditions that simulate those under which consumers make their selections. For meat this includes retail cases, lighting, case and meat temperature, defrost cycling, overwrap and packaging (AMSA 1991). Some foods, such as hot brewed tea or frozen desserts, change appearance quickly. Hence special viewing arrangements for the panel are necessary even to the extent of having panellists file around a table scoring each sample as they pass (Hutchings 1999).

Where assessments take place on a production line, good consistent conditions for viewing the sample or matching to a memorised or formal colour standard need to be made available. Market researchers often find themselves in conditions unsuitable for the making of reliable appearance assessments. A specification of the environment of the test, including types of illumination and colour of the test surrounds, should be noted in the final report. This will make it easier for the occurrence of colour metamerism problems to be minimised. If the consumer's views of product colour and appearance are important to the project, standard lighting and viewing conditions should be provided.

## 2.14 Lighting for appearance assessment

The light by which we view food materials is critical to an assessment of appearance. Many types of lighting installation operating over a wide range of intensities are to be found in development, manufacturing, retail, preparation and dining areas. Appropriate light sources and levels are essential for obtaining reproducible, reliable assessment results. Lighting of panel booths is of concern in two ways. It may be used to provide illumination for quality assessment but it also may be required to hide sample colour.

Choice of lighting installation for colour assessment depends upon the products and needs of the concern, and on the illumination used by customers in their assessment rooms, display areas, dining rooms or shops. Constant, extended area, controllable incandescent and/or fluorescent light sources are required to provide even illumination free from shadows. It should be sufficiently directional to allow perception of textured surfaces. Extraneous light sources should be excluded from the booth area. For colour assessment, specular reflection of the source (that is, where the angles of incidence and reflection are approximately the same) should be minimised. This can be achieved by ensuring that the light source is vertically above, and the viewing takes place at approximately 45 degrees to the sample.

Dimmable systems capable of operating between approximately 750 lux, the level recommended for small offices (van Ooyen *et al.* 1987), and 1200 lux are

recommended (Eggert and Zook 1986). An intensity of 807 to 1614 lux is recommended for evaluation of fresh meat (AMSA 1991). The British Standard BS950: part1 is concerned with defining the spectral distribution of a light source of correlated colour temperature 6,500 K, which can be used to replace daylight for visual appraisal and colour matching tasks (BS 1967). No practical source exactly meets this standard. However, the Artificial Daylight tube, which includes an ultraviolet component, and Northlight, which does not contain such a component, are currently regarded to be the closest approximations available. These are widely used in industries in which colour assessment is critical. Some metal halide and fluorescent lamps having high colour rendering indices are used for assessment in the laboratory. Wherever possible product assessments should take place under the same conditions used by the customer. Specific viewing conditions are recommended for using colour atlases (USDA 1975). For general evaluation of intermediate lightness colours the intensity should be between 810 and 1880 lux. The spectral quality of the light should approximate to that of daylight under a moderately overcast sky at a colour temperature of 6770 K, equivalent to Illuminant C, or 6500 K, equivalent to D65; direct sunlight should be avoided (BS 1999, ASTM 1994, USDA 1978).

The assessment of gloss in sensory booths can be difficult because of the area, lighting, and angular viewing needed for its perception, and alternative arrangements may be needed. In some industries a specially designed lamp is used for visual evaluation of gloss differences. This lamp consists of a desk fluorescent lamp, which has a black reflecting surface behind the tube and a wire mesh grid in front (ASTM 1985). This lighting allows the observer to assess any of the several types of gloss occurring in foods.

For strict judgements of flavour and texture variations in appearance must be eliminated from view. Very low levels of coloured lighting are often used for this but this tactic often fails in its objective. For example, neither dark red, blue, green or mauve light nor sodium lighting are successful at masking colour differences of orange juices, hence judges wore blindfolds (Barbary *et al.* 1993). Half-filled dark-coloured glasses are suitable for fluids. Alternatively the sample can be presented in total shadow by illuminating the opaque sample container from beneath. Even so, light from extraneous sources can increase ambient levels to an unacceptable extent. The possibility of all such effects must always be taken into consideration.

Lighting in the briefing room should be the same as that under which samples are to be viewed. This enables each panellist to become visually adapted to booth viewing conditions. When lighting is changed during the experiment time must normally be allowed for the subject to become adapted. If doors are included at the back of each booth precautions are needed to prevent the introduction of lighting inconsistent with that inside the booth. If this cannot be achieved samples should be introduced through a light baffle.

Lighting installations should be ventilated, especially when tungsten illumination is provided, and all units should be regularly checked. Lighting levels and colour temperature should be monitored and lamp replacements made

as recommended by the manufacturer. Filters and light units need regular checking and cleaning every three months when the units are cool, again as specified by the manufacturer. A maintenance log should be used.

## 2.15 Appearance profile analysis

Quality judgements are controlled not merely by colour but by total appearance. This consists of the visually assessed:

- structure, that is, the geometry and structural including blemishes
- surface texture, of each structural element
- colour, translucency, gloss, of each structural element
- colour, translucency, gloss uniformities and patterns of each structural element
- temporal properties, that is, those features that change with time, spatially, or with ageing.

This forms the basis of appearance profile analysis (APA) which can be used to catalogue logically such properties (Hutchings 1999). The technique can be used to define and understand product appearance, as well as customer response to appearance. It provides the methodology for development of designer products and the scientific basis upon which product appearance development can be promoted.

In illustration we can consider a custard dessert topped with cherries. Many appearance factors may be important to the selling success of such a product. There are the visual properties of the dish as a whole including the container, the visual properties of each component of the dish, and the contrasts and relationships between each component. That is, the custard and cherries have complementary as well as individual attributes. These include the perceived volume of the whole dish and the perceived volume contrast of each component, the symmetry or randomness of position of the cherries, their number, size, wholeness and defects, the perceived colour and colour uniformity of each component and their colour contrast, the perceived and contrast translucency and gloss of each component, and the perceived texture and texture contrast of both custard and cherries. In such cases, an APA reveals the properties of the product as a whole as well as of each component in sufficient detail to make disciplined comparative judgements between two products or between product and concept. These are the basic perceptions.

Expectations or derived perceptions of visual safety, visual identification, visual flavour, visual texture and visual satisfaction are deduced and derived from appearance. For the cherry custard they include identification of its place within the meal, that it is a dessert, as well as provoking some level of satisfaction appropriate to the observer. Additional expectations include:

- for the custard – lumpiness, thickness, yellowness, vanillaness
- for the cherries – cherriness, redness, transparency, sourness
- for the dessert as a whole – tastiness, sweetness, fillingness, sufficiency for present needs, appetisingness, wholesomeness.

Subjects using connotative scales can assess such expectations. For example, a lumpiness scale ranging from very smooth to very lumpy can be devised for the custard. The relationships between basic and derived perceptions reveal the appearance and expectation details for the customer in the shop, cook in the kitchen and diner in the restaurant.

In many cases it is not necessary to make a formal statement of the complete analysis. A decision may be reached as to the most important appearance aspects for a particular product in a particular situation, and other attributes may be eliminated from further consideration. However, this attribute elimination ought to be made positively on an understanding of the appearance science of the product, not by neglect. Use of basic perception sensory descriptors and scales is straightforward only when discussion, agreement, training, practice, and continuing retraining are conscientiously undertaken. Derived perceptions are obtained from the consumer.

## 2.16 Future trends

Global marketing means that understanding of peoples becomes ever more pressing and there is much to be learned about the creation of expectations from product appearance. As indicated in this chapter the foundation has been laid. Current research is increasing understanding of neural perception mechanisms and revealing how specific sensations are recognised and recorded.

The ability to perform precise, calibrated digital measurements is a welcome breakthrough for the science and technology of food appearance. As well as a new future for the measurement of appearance properties of three-dimensional materials it will be of great benefit for sensory assessment. Potential benefits include the development of on-screen panelling and panel manipulation, communication and archiving. Another benefit will be the construction of improved colour standards for panel use (see Chapter 14).

## 2.17 Sources of information and advice

In the food business there are many applications for intervention of appearance science – examples are the product itself, consumer expectations, advertising and packaging, marketing. Studies of food colour psychology, folklore and anthropology are available (Davidoff 1991, Hutchings *et al.* 1996, Meiselman 2000).

This book will add considerably to currently available information on food colorants and conventional colour measurement. A wider look at the philosophy

of food appearance, appearance sensory science applied to the food business and the measurements of all aspects of appearance is available (Hutchings 1999). Expectations in the food industry have also been studied (Hutchings 2001).

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# 3

## Colour measurement of food

### Principles and practice

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#### 3.1 Introduction

We perceive the world in which we live by our five senses, vision, hearing, touch, taste and smell, of which the sense of vision is usually the first used in detecting events and objects around us in the visual world. The process of seeing comprises many co-operating activities, first detected by our eyes and then interpreted in our brain, recognition of movement and location of object, relationships of objects to their surroundings, the intensity and quality of the light and the colour appearance of objects or events in the visual scene. From the time humans first recorded events pictorially, e.g., cave paintings and then by printing, the incorporation of colour as a medium was an integral component of the procedures. This was especially so in the development of printing and in the world of art. Artists, from earliest times, attempted to portray the colour appearance of food in their pictures as realistically as possible with the limited number and type of pigments available. Most national art galleries contain examples of both classical and modern painting where food items are part of or are central to the painting. With modern photographic techniques and computer-controlled printing, accurate and attractive pictures of food items are now expected in illustrated magazine articles. Although representational portrayal of natural objects by paint and print can appear real and visually pleasing, it was only in the last two centuries or so that a scientific understanding of the processes involved in determining colour appearance has been elucidated.

Scientific studies into the mechanism of vision and human colour perception began in the seventeenth century with the recognition that the eye's lens must somehow project an image of the object viewed onto the back of the eye. Newton's classic experiments on the refraction of light led him to conclude that



the rainbow did not possess colour but it was the spectrum's rays that produced the sensation (Wright 1967). The rationality of arranging colours into orderly systems, based on Newton's seven rainbow colours, has resulted in the construction of colour atlases which attempt to arrange their colours in such a way that equal visual distances exist between adjacent colours. Two of the most used atlases are the earlier Munsell system and the newer Swedish natural colour space system. The former, developed in the USA, is based on five hues and the latter, used mainly in Europe, is based on the six unique perceptions of black, white, red, green, yellow and blue (Hard and Sivik 1981).

The experiments of Maxwell, Young and Helmholtz in the nineteenth century in mixing coloured lights (MacAdam 1970) clearly demonstrated that people with normal colour vision must have at least three retinal pigments in their eyes, detecting in the short-, mid- and long-wave regions of the visible spectrum. By the late 1920s and early 1930s, the eye's sensitivity to light relative to wavelength was established and the so-called 'standard observer' defined (Wright 1980). This led to the first truly functional system for measuring colour as specified by the Commission Internationale de l'Éclairage (CIE), the so-called CIE 1931 2° visual field system of colour measurement (CIE 1986). Although colours could be defined unambiguously in this space, the space is not visually uniform. Since that time, many improvements have been incorporated into the system to make it nearly visually uniform and this research continues. With the development of the computer, complex colour measurements and calculations are now routinely used for such industrial processes as paint formulation (Best 1987), colour match prediction (Nobbs 1997) and control of the appearance of dyed textiles (McLaren 1986; McDonald 1997). Improvements in instrument specification and design have led to a considerable increase in their use in industry. In the food industry, colour measuring instruments are now routinely used in research and for studies into product functionality, for product ingredient standardisation and process control.

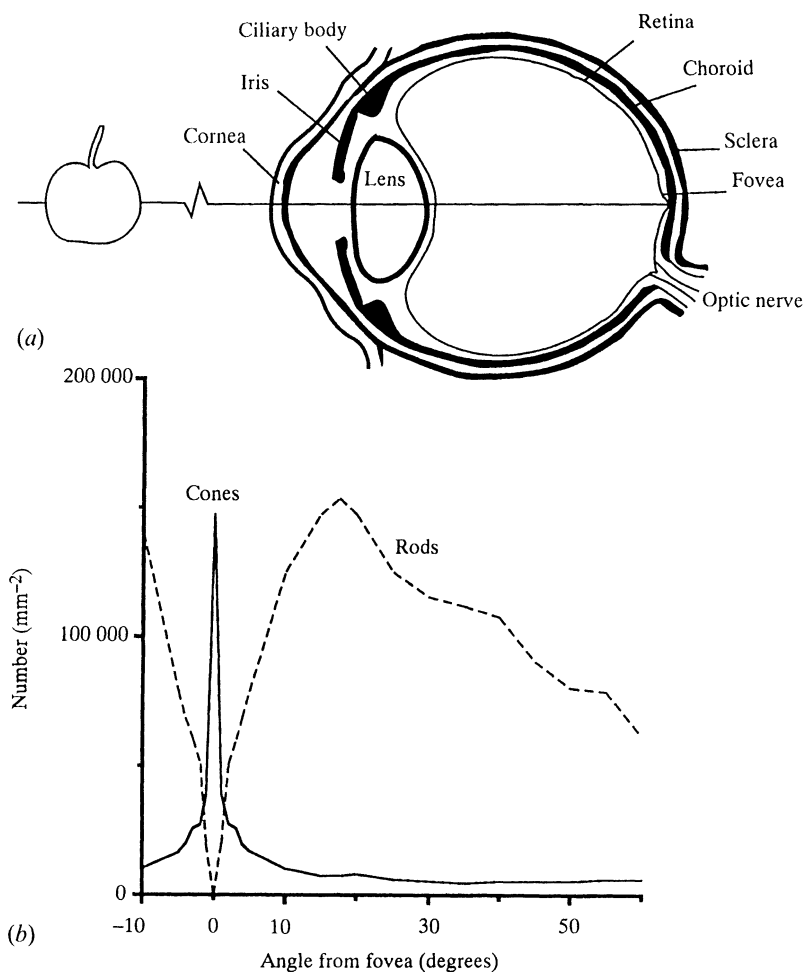
### **3.2 Colour vision: trichromatic detection**

Three interacting factors are required for the measurement of the colour appearance of any object in a scene. These are an understanding of the human visual process, the effect of light on objects in their environment and the nature of the materials observed. The sensation of colour is a psychophysical phenomenon, which is only part of the overall visual perception of the information detected by the eye and interpreted by the brain. The complex visual response can be thought of as the sum of the responses recognised in the brain from the signals detected by the eye of the scene viewed. The sensation, therefore, is perceived as if it were projected out into the world from which it originated. This leads to the error of imputing to the scene the sensations it generated. Sensations exist in the observer's mind and not in the external world which produces them.

Human eyes have a near circular field of view and are composed of three membranes (Fig. 3.1a). The outer membrane, the sclera, is continuous posteriorly with the sheath of the optic nerve and anteriorly with the cornea. The iris and the ciliary body, which suspends the lens, arise out of the middle layer, the choroid, which contains the capillary network. The light-detecting inner membrane, the retina, lines the inside of the posterior of the eye. The first step in the visual process is the automatic control of the amount of light entering the eye through the iris. The flux is then focused by the lens on to the fovea in the central region of the retina where it is detected as colour. The signal is amplified (Normann and Werblin 1974) and then transmitted through the visual pathway (Rodieck 1979) for interpretation in specific areas of the visual cortex of the brain (Hubel 1988; Zeki 1993). The retina has two types of light-detecting receptors, the cones and rods, so named because of the shape of their structures as viewed by the microscope. The 'photopic' colour-detecting cones are sensitive to three wavelength ranges in people with normal colour vision and are densely packed in mosaic pattern in the centre of the fovea. This occupies  $< 2^\circ$  of the visual field and is the basis of the CIE 1931  $2^\circ$  standard colorimetric observer. The  $>100$  times more sensitive 'scotopic' colourless detecting rods increase in density to  $20^\circ$  from the fovea and then decrease towards the periphery of vision (Fig. 3.1b). In 1964 a supplementary standard observer with a  $10^\circ$  field of view was created to accommodate the changes that occur in colour perception as the visual angle increases beyond  $2^\circ$  where some rods are included in the detecting field.

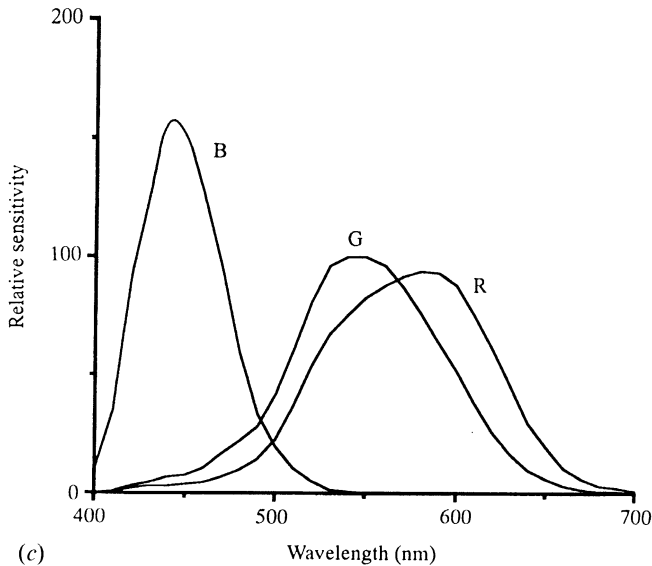
Light energy, focused on the retina, is converted into electrical signals by changes in the conformation of the photopigments in the multifolded disk-shaped structures in the outer segments of the rods and cones (Wald 1968; Hurvich 1981; Jacobs 1981; Stryer 1988). Although only the rod pigment rhodopsin has been characterised, determination of the spectral absorption of the cone pigments has been possible using retinal densitometry with colour-blind observers deficient in one pigment (Smith and Pokorny 1975). The sensitivity curves of human cones determined by Estévez (1982) are presented in Fig. 3.1c. Rhodopsin absorbs maximally at 505 nm and the so-called blue (B), green (G) and red (R) cone pigments, that is the short-, mid- and long-wave sensors, are maximally sensitive at approximately 440, 540 and 570 nm respectively. The sensitivity range of B absorption marginally overlaps G and R between 450 and 550 nm, whereas the G and R absorption functions overlap substantially, displaced from each other by only 20 to 30 nm. Thus monochromatic light at 580 nm which is near maximum for R appears yellow and not red because of the combined contributions of G and R. Increasing the wavelength to  $> 600$  nm increases the contribution of R relative to G and the perceived colour becomes redder. The fact that the R and G cones overlap gives rise to the enormous number of colours and colour differences that are experienced by people with normal colour vision.

Because cone vision is trichromatic, it means a suitable mixture of red, green and blue primary lights, as would be expected from a three-receptor system, can



**Fig. 3.1** Detection of light in the eye: (a) structure of the human eye (b) distribution of cones and rods in the temporal side of the retina (nasal side is similar except for the blind spot between 12° and 18° from the fovea).

match any coloured light. The actual colour-matching functions depend on the wavelengths used as primaries. Although the initial detection of the stimulus is trichromatic, subsequent post-retinal processing gives rise to an achromatic lightness/darkness mechanism and coloured red/green and blue/yellow opponent mechanisms (Hurvich 1981; Hunt 2001). The lightness component consists of a weighted summation of all three cone pigment absorptions, whereas it is the degree of differences among the B, G, R absorptions that generates the opponent colour mechanism. However, the neural linkages among the pigment cone signals are not in simple one-to-one opposition. The simplest scheme that can be constructed is that the red/green opponent response is red activated by



**Fig. 3.1** continued. (c) spectral sensitivities of blue (B), green (G) and red (R) cone pigments.

absorption of B plus R and green activated by G; yellow is activated by G plus R in opposition to blue activated by B.

### 3.3 The influence of ambient light and food structure

#### 3.3.1 Adaptation and colour constancy

Adaptation is the process whereby the visual system conditions itself to the chromatic nature of the surroundings as affected by the quality, that is the wavelength distribution, and intensity of the illumination. It compensates for changes in the spectral power distribution of the light and serves to keep the eye in balance (Boynton 1979). The magnitude of this near automatic adjustment that chromatic adaptation has on visual experience is not usually recognised because of the limitations of human memory for individual colours and the phenomenon of colour constancy (Brill and West 1986). White objects appear to be white over a vast range of light conditions, e.g., from bright sunlight to the relatively dim levels of light found in room interiors, while colours appear to have similar colour appearance under most types of white or near white illumination. Studies into the phenomena of adaptation which elicit this near constancy of colour appearance have been concerned mostly with predicting the changes that occur to colour recognition when lamp type and output are altered (Bartleson 1979a). Lightness and contrast among greys are affected by luminance while colourfulness increases with increase in the level of illumination and varies with the spectral emission of the lamp and its colour

temperature (Hunt 1977). The phenomena of adaptation can be subdivided into three components, chromatic adaptation, light adaptation and colour constancy.

Chromatic adaptation occurs where changes in the visual system compensate for changes in the spectral quality of the illumination. Light adaptation occurs where the visual system attempts to compensate for changes in the level of illumination and colour constancy is experienced where the colour of an object tends to remain constant although the level and colour of the illumination are changed (Berns 2000). This is further discussed in Section 3.9.1 on fresh meat, where the degree of red enhancement of lamp spectra is shown to affect the perception of product attractiveness. Models of cone adaptation response have been used to predict the consequences of changing lamp spectra on object appearance (Bartleson 1979b; Nayatani *et al.* 1986; Hunt 1987). The concept of apparent colourfulness has been used to construct grids of constant hue from which other grids can be derived for other illuminants (Pointer 1980; 1982). Such models use logarithmic and hyperbolic functions to mimic the physiological mechanisms involved. Hunt's (1987) model can be used to predict the changes that occur in object colours at any level of illumination for a wide range of backgrounds in the realistic situation where the eye's fixation wanders. A considerable amount of research has been done into the subject of chromatic adaptation since the late 1980s. The most recently tested chromatic adaptation transform, CMCCAT2000, for predicting the change in colour appearance on changing illuminant has been shown to be simpler to use than previous models and has superior predictive accuracy (Li *et al.* 2002). These effects of light quality on colour perception illustrate the difficulties in separating the concept of vision from that of appearance. The light from the scene modulates vision, whereas the characteristics of appearance are modified by the light incident upon the object. The concepts of colour constancy and adaptation are discussed further in Chapter 4 of this book. Hence, procedures devised for observing and measuring colour must take account of the nature, quality and quantity of the light as it affects the observer's perception. The British Standards Institute and the International Standards Organisation have recently produced general guidance and test methods for the assessment of the colour of foods (BSI 1999)

### 3.4 Appearance

Colour is usually considered the most important attribute of any food's appearance (Francis and Clydesdale 1975) especially if it is associated with other aspects of food quality, for example, the ripening of fruit or the visible deterioration which occurs when a food spoils. Nearly every food product has an acceptable colour range, which depends on a wide range of factors including variability among consumers, their age and ethnic origin, and the physical nature of the surroundings at time of judgement (Francis 1999). However, in addition to colour specification, the nature and extent of internally scattered light and the

distribution of surface reflectance are required for a more complete description of appearance. The food's structure and pigmentation interact to affect both colour and translucency/opacity, for example, small changes in scatter may produce larger changes in colour appearance than are attributable to change in pigment concentration (MacDougall 1982).

The characterisation of an object's appearance is accomplished in two stages. The first is physical and the second is psychological. The physical characteristics are the size, shape and uniformity of the object along with the type, degree and variability of pigmentation throughout the object and the nature of the object's structure that attenuates light. The physical information is converted to the psychological by translating the object's reflectance or transmittance spectrum into its tristimulus values and then to a defined colour space.

The concept of 'total appearance' (Hutchings 1999) can be applied to foods and is further discussed in Chapter 2 of this book. Total appearance comprises more than just the food's physical appearance characteristics and takes account of such social factors as the observer's culture, memory, preferences and appreciation of the product. Foods have an infinite variety of appearance characteristics. Their surfaces may be diffuse, glossy, irregular, porous, or flat. They may be transparent, hazy, translucent or opaque and their colour may be uniform, patchy or multilayered. Hence, colour-measuring procedures for foods often have to be modified from those used in the measurement of flat opaque surfaces such as paint and paper, for which most colour-measuring instruments are designed. However this is not always recognised by those involved in food colour appearance measurement. Different instrument optical geometries will lead to difficulties in sample presentation and, coupled with the uncertainties of sample structure, are likely to give different colour values for the same material if measured on different instruments. The inclusion or exclusion of surface specular reflection in the measurement procedure depends not only on its importance as a characteristic of the food but also on the design of the detector-sensor unit in the instrument.

The spreading of the light transmitted within the food depends largely on its structure as well as the level of pigmentation. Hence, lateral transmittance of light through translucent materials will affect both their reflectance and visual appearance (Atkins and Billmeyer 1966; Hunter and Harold 1988; MacDougall 1988, Hutchings 1999). The translucence effect must be allowed for in the assessment of such products as tomato paste (Brimelow 1987) because the ratio of absorption to scatter varies with aperture area and the concentration of components in the product (Best 1987; MacDougall 1987).

### **3.5 Absorption and scatter**

The reflection of light from opaque and translucent materials depends on the ratio of absorption to scatter as affected by pigmentation, refractive index and the light-scattering properties of the material. The Kubelka-Munk (KM) method

for separating subsurface absorption and scatter (Kubelka 1948) is illustrated by Judd and Wyszecki (1975). Its use in the determination of pigment absorption in opaque materials is given in the latest edition of the book by Billmeyer and Saltzman (Berns 2000) and its use in opaque, translucent and layered materials is fully discussed by Nobbs (1997). The KM procedure relates reflectivity  $R_\infty$ , i.e., reflectance at infinite thickness, to the coefficients of absorption  $K$  and scatter  $S$  by

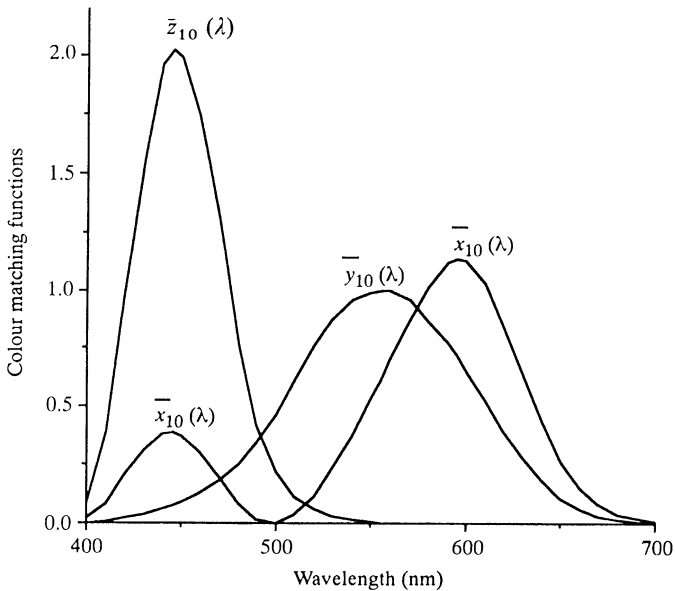
$$K/S = (1 - R_\infty)^2 / 2R_\infty$$

Hence  $K/S$  can be calculated directly from measurement of infinite thickness but to calculate  $K$  and  $S$  separately it is necessary to measure the reflectance of thin layers mounted on white and black backgrounds. If  $K$  and  $S$  are required for prediction purposes, the accuracy of their measurement can be improved by appropriate correction factors for surface reflection (Saunderson 1942). Colour calculated from  $R_\infty$ , with separate estimation of the specular component as gloss is usually sufficient information to describe opaque objects, but for translucent or layered materials  $K$  and  $S$  are also necessary.

### 3.6 Colour description: the CIE system

The CIE system of colour measurement (ASTM 2000, CIE 1986) transforms the reflection or transmission spectrum of the object into three-dimensional colour space using the spectral power distribution of the illuminant and the colour-matching functions of the standard observers (CIE 1986). The mathematical procedures are given in any standard text on colour, for example Wright (1980), Judd and Wyszecki (1975), Hunt (2001) and Berns (2000). The system is based on the trichromatic principle but, instead of using 'real' red, green and blue primaries with their necessity for negative matching, it uses 'imaginary' positive primaries X, Y, and Z. Primary Y, known as luminous reflectance or transmittance, contains the entire lightness stimulus. Every colour can be located uniquely in the 1931 CIE colour space by  $Y$  and its chromaticity co-ordinates  $x = X/(X + Y + Z)$  and  $y = Y/(X + Y + Z)$ , provided the illuminant and the observer are defined.

The original illuminant representative of daylight was defined by the CIE as source C, but is now superseded by D65, i.e., an illuminant which includes an ultraviolet component and has a colour temperature of 6500°K. The colour temperatures of lamps and daylight range from approximately 3000°K for tungsten filament lamps and 4000°K for warm white fluorescent to 5500°K for sunlight and 6500°K for average overcast daylight to approximately 20000°K for totally sunless blue sky. Because the original 2° colour-matching functions apply strictly only to small objects, i.e., equivalent to a 15 mm diameter circle viewed at a distance of 45 cm, the CIE has added a 10° observer (Fig. 3.2) where the object diameter is increased to 75 mm. Currently, the trend in colour



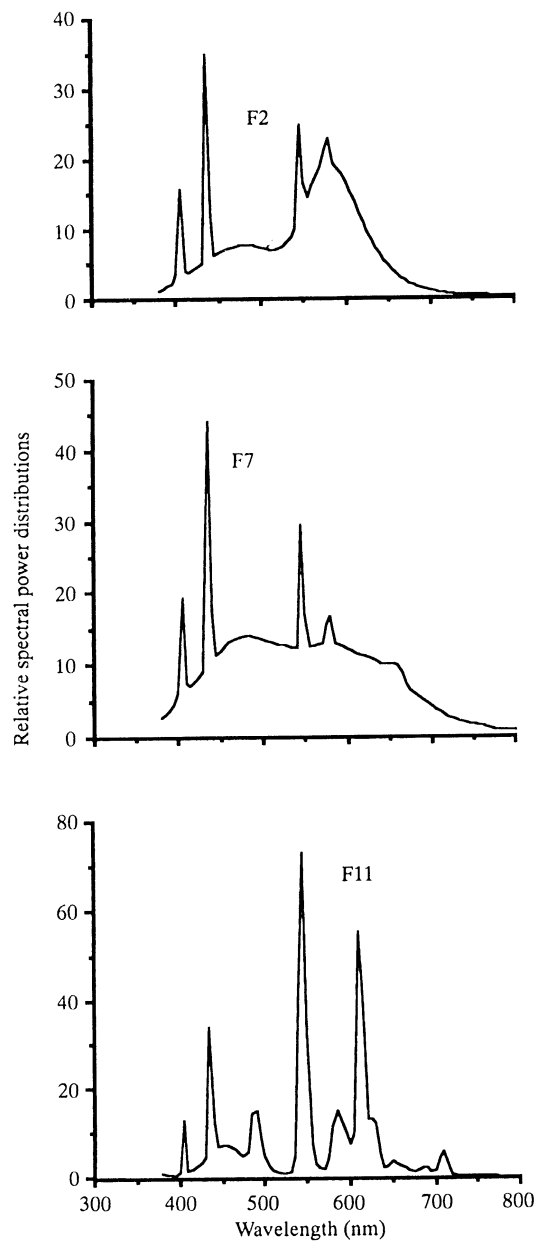
**Fig. 3.2** Colour matching functions of the CIE 10° standard observer.

measurement is to use D65 and the 10° observer except for very small objects. The 1986 CIE recommended procedures for colorimetry are included in the ASTM Standards (2000) and also in Hunt (2001) along with the weighting factors for several practical illuminants (Rigg 1987). These include representative fluorescent lamps, of which F2 is a typical lamp at 4230° K but with a low colour-rendering index ( $R_a$ ) of 64 (Fig. 3.3). The colour-rendering index  $R_a$  is a measure of the efficiency of a lamp at a given colour temperature to render the true appearance of Munsell colours. The broadband lamp F7 has the same colour temperature (6500°K) and chromaticity co-ordinates as D65 and, because of its flatter spectrum, it has a high  $R_a$  of 90. The triband lamp F11 (4000° K) also has a moderately high  $R_a$  of 80, but its main advantage is its much improved efficiency in energy utilisation.

### 3.7 Colour description: uniform colour space

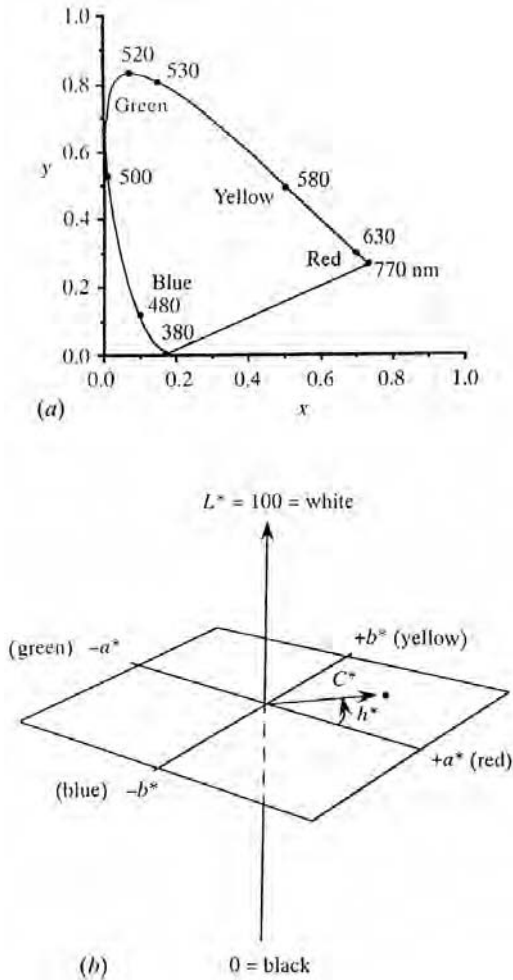
The original 1931 CIE Y, x, y system of colour measurement is not visually uniform (Fig. 3.4a). Constant hue and chroma are distorted and equal visual distances increase several-fold from purple-red to green. Improved spacing has been accomplished by both linear and non-linear transformations of Y, x, y (Berns 2000). Near uniform colour spaces of practical importance are the Hunter and the CIELUV and CIELAB spaces. In the Hunter (1958)  $L, a, b$  colour space the lightness co-ordinate  $L$  is the square root of the tristimulus value  $Y$ , and  $a$ , and  $b$  are the red/green and yellow/blue opponent co-ordinates. The 1976





**Fig. 3.3** Relative spectral power distributions of preferred CIE representative fluorescent lamps.

CIELUV and CIELAB spaces (Robertson 1977) attempted to reduce the many scales then in use to two. The lightness co-ordinate  $L^*$  is the same for both but the spaces use different concepts in their construction. The CIE  $L^*, a^*, b^*$  space



**Fig. 3.4** Colour diagrams: (a) CIE 1931 chromaticity diagram showing non-uniformity of spacing of red, yellow and blue unique hues; (b) CIELAB uniform diagram showing relationship of red/green ( $a^*/+/-$ ) and yellow/blue ( $b^*/+/-$ ) opponent co-ordinates to lightness  $L^*$ , chroma  $C^*$  and hue angle  $h^*$ .

(Fig. 3.4b), known as CIELAB, has generally replaced the Hunter space for industrial applications although this has been somewhat slower in parts of the food industry where methods established on the Hunter system have economic reasons for its continued use. The improvements in CIELAB are due to the non-linear cube root transformation of the 1931 tristimulus values, which more approximate the visual spacing of the coloured samples in the Munsell system. The formulae are

$$L^* = 116(Y/Y_n)^{1/3} - 16 \quad \text{for } Y/Y_n > 0.008856$$

$$L^* = 903.3(Y/Y_n)^{1/3} \quad \text{for } Y/Y_n < 0.008856$$

$$a^* = 500[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}]$$

$$b^* = 200[(X/X_n)^{1/3} - (Z/Z_n)^{1/3}]$$

where  $X_n$ ,  $Y_n$ ,  $Z_n$  refer to the nominally white object colour stimulus.

The co-ordinates of  $L^*$ ,  $a^*$  and  $b^*$  in CIELAB serve to define the location of any colour in the uniform colour space. However, in most industrial applications the object of measuring products is usually to determine how far they may be divergent from a set standard, both in colorimetric terms and in acceptability of visual match. The determination of uniform colour differences by CIELAB is not the same as the recognition of acceptability. CIELAB is based on the perception of just noticeable colour differences in the cylindrical co-ordinates of the system. However, acceptability differences are based on the perception of colour tolerance differences of real materials of industrial interest, e.g., textiles.

Colour terms can be divided into the subjective and the objective (Hunt 1978). The subjective, i.e., the psychosensorial, are brightness, lightness, hue, saturation, chroma and colourfulness. Colourfulness, a more recently introduced term, is that aspect of visual sensation according to which an area appears to exhibit more or less chromatic colour. Although hue is easily understood as that attribute described by colour names red, green, purple, etc., the difference between saturation and chroma is less easily comprehended. Saturation is colourfulness judged in proportion to its brightness, whereas chroma is colourfulness relative to the brightness of its surroundings. A similar difference exists between lightness and brightness. Lightness is relative brightness. Lightness is unaffected by illumination level because it is the proportion of the light reflected, whereas the sensation of brightness increases with increase in the level of illumination.

The objective, i.e., the psychophysical are related to the stimulus and are evaluated from spectral power distributions, the reflectance or transmittance of the object and observer response. They provide the basis for the psychometric qualities which correspond more nearly to those perceived. For CIELAB space the terms are lightness  $L^*$ , hue  $h^* = \tan^{-1}(b^*/a^*)$  and chroma  $C^* = (a^{*2} + b^{*2})^{1/2}$ . CIELAB total colour differences  $\Delta E^*$  can be expressed either as the co-ordinates of colour space or as the correlates of lightness, chroma and hue. Hence

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

or

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta C^*)^2 + (\Delta H^*)^2]^{1/2}$$

where  $\Delta H^*$  is used rather than  $\Delta h^*$  because the latter is angular. For small colour differences away from the  $L^*$  axis, if  $h^*$  is expressed in degrees, then

$$\Delta H^* = C^* \Delta h^* (\pi/180)$$

The major colour scales with their associated terminology are given in Table 3.1.

It has been the task of the CIE for several years to create a single number pass/fail equation that would weight the three components that make up the total CIELAB colour difference  $\Delta E^*$ , that is,  $\Delta L^*$ ,  $\Delta C^*$  and  $\Delta H^*$  the lightness,

**Table 3.1** Overview of colour description systems and notation

*CIE system (1931)*

This is based on the imaginary positive primaries  $X$ ,  $Y$ ,  $Z$  (transformed from real red, green and blue trichromatic primaries which may contain negative values).

In CIE space, colour is located by  $(Y, x, y)$ , where

- $Y$  luminous reflectance or transmittance (containing the entire lightness stimulus)
- $x, y$  chromaticity co-ordinates
- $x = X/(X + Y + Z)$
- $y = Y/(X + Y + Z)$

CIE space is not visually uniform.

*Hunter Lab System (1958)*

In Lab space, colour space is more uniform than CIE and is defined by  $(L, a, b)$ , where

- $L$  correlate of lightness
- $a, b$  red/green and yellow/blue opponent co-ordinate correlates
- $L = 10 Y^{1/2}$
- $a = [17.5(1.02X - Y)]/Y^{1/2}$
- $b = [7.0(Y - 0.847Z)]/Y^{1/2}$

*CIELAB system (1976)*

In CIELAB space, colour space is defined by  $(L^*, a^*, b^*)$ , where

- $L^*$  visually uniform lightness
- $a^*, b^*$  visually uniform chromaticness co-ordinates

$$L^* = 116(Y/Y_n)^{1/3} - 16 \quad \text{for } (Y/Y_n)^{1/3} > 0.008856$$

$$L^* = 903.3(Y/Y_n)^{1/3} \quad \text{for } (Y/Y_n)^{1/3} < 0.008856$$

$$a^* = 500[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}]$$

$$b^* = 200[-(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}]$$

Where  $X_n, Y_n, Z_n$  are the values of  $X, Y, Z$  for the reference white.

Further terms used are

$$h^* = \tan^{-1} (b^*/a^*) \quad \text{hue}$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad \text{chroma}$$

Note: Recent colour difference formulae CMC(1;c) and CIE94 are derivations of CIELAB with weighting functions applied to make vectors of  $\Delta L$ ,  $\Delta C$  and  $H$  more visually acceptable. The most recent and superior formula is CIE2000. Worked example of CIE2000 is given in Luo *et al.* (2001) The most recent chromatic adaptation formula for describing colour appearance under different viewing conditions is given in Li *et al.* (2002)

chroma and hue vector differences. Equations that are a distinct improvement in this regard have been devised by the CIELAB on a very large body of experimental evidence (Berns 2000). The Colour Measuring Committee (CMC) of the Society of Dyers and Colourists formulated a significant improvement in uniform colour difference formulae from the earlier JPC79 (J and P Coates) colour difference formula. In this CMC(l:c) formula, adjusting constants are incorporated by the user to weight the importance of lightness and chroma relative to hue (BSI 1988). Subsequently, on the basis of the work of Luo and Rigg (1986), Alman *et al.* (1989) and Berns *et al.* (1991), the CIE recommended the use of a new colour difference equation for use in industry, known as CIE94 where total colour difference is designated as  $\Delta E_{94}$ . It includes a term for the visually perceived magnitude of the colour difference. For those industries that require accurate colour difference measurement that is related to perception and acceptability, e.g., the textile industry  $E_{94}$  is used preferentially. A further improvement has now been recommended by the CIE on the basis of work described by Luo and his associates and is designated as the CIEDE2000 Colour-Difference Formula (Luo *et al.* 2001). It has now been officially adopted by the CIE (CIE 2001) and a worked example of its use is given in the Luo *et al.* (2001) publication. The food industry's demand for such a level of precision of colour difference as recommended by CIEDE2000 remains to be assessed.

### 3.8 Instrumentation

Since colour is a psychological phenomenon, its measurement must be based on human colour perception. Hence, photoelectric instruments are corrected for both lighting and human visual response, while visual techniques must use observers with 'normal' colour vision under defined lighting. Examples of direct visual assessment are colour atlases for broad definition of the location of colours in colour space, collections or sets of printed or painted coloured papers specific to products or processes and visual matching instruments which use coloured filters. Typical of the former are the Munsell and Swedish NCS atlases which are structured on uniform colour space, and the Pantone collections of printer's colours with defined ink mixtures printed from 10 to 100 per cent tinting strength. Probably the best known of the visual matching instruments is the Lovibond Tintometer in which the object, under specified illumination, is viewed and matched against a series of coloured filters interposed over a white background by the observer.

Photoelectric colour measuring instruments can be divided into two classes, trichromatic colorimeters and spectrophotometers. The most successful of the early trichromatic colorimeters was developed in the 1940s by Hunter (1958). It comprised a light source and three wideband red, green and blue filters to approximate CIE standard illuminant C and the 2° observer. The tristimulus values obtained were transformed into Hunter L, a, b colour space. Until the advent of the computer and the photodiode such

instruments were much less expensive than spectrophotometers and, although absolute accuracy may have been poor, they were extremely good at measuring the small colour differences demanded for industrial process control (Patterson 1987). The more modern tristimulus instruments are linked to computers with automatic calibration and the provision of a number of colour spaces. Such instruments may be supplied with a selection of sensor heads of different illuminating geometries to allow measurement of a wide range of product types depending on the nature and dimensions of their surfaces. Several companies now manufacture a range of hand-held lightweight colorimeters and miniature diode array spectrophotometers, with optical geometries comparable in function with the larger bench instruments. Their compactness is a direct result of the use of high-energy pulsed xenon arc lamps combined with filtered silicon detectors and microchip circuitry. Because such instruments, with their built-in memories and choice of colour scales, are comparatively inexpensive it has resulted in an increase in their use for in-line colour measurement in all branches of industry where colour control is necessary or desirable, e.g., in the printing and automotive industries.

The most accurate instrument for measuring colour is the spectrophotometer. Reflectance instruments are usually fitted with an integrating sphere with the choice of including or excluding the specular component of reflectance. Care must be exercised in deciding which geometry is appropriate for particular applications. The diffuse component of reflectance from subsurface absorption and scatter is wavelength dependent, whereas the specular component is not. For materials with glossy surfaces the inclusion of the specular will increase measured reflectance which, when translated into colour space, can lead to large discrepancies in the interpretation of visual lightness, as usually viewed, and to a lesser extent of the chromaticness of the colour. For example, highly glossy black tiles used for instrument calibration have tristimulus  $Y$  values of approximately 0.3 when the specular is excluded but 4.5 when included. The consequence of this difference in  $Y$  of 4 per cent produces a specular excluded uniform lightness  $L^*$  of 3 and an included  $L^*$  of  $> 25$ . For medium grey and white tiles the excluded to included  $Y$  values are approximately 25 to 29 and 78 to 82 respectively, which give  $L^*$  values of approximately 57 to 61 and 91 to 92 respectively. Hence the near constant effect of 4 per cent in  $Y$  from the specular reflectance produces a decreasing effect from black to white from  $> 20$  to about 1 per cent in  $L^*$ . The CIE recommends that colorimetric specifications of opaque materials should be obtained with one of the following conditions of illumination and viewing geometries which should be specified in any report:

- $45^\circ/0^\circ$  or  $0^\circ/45^\circ$ , specular excluded
- diffuse/ $0^\circ$  or  $0^\circ$ /diffuse, specular included or excluded.

However, spectrophotometers most commonly used for measuring colour do not have identical geometries. Three typical instruments were compared by Patterson (1987), who points out that probably the biggest source of differences

among the instruments can be traced to the specular component. Hunt (1987) suggests that if measurements are to be compared it is better to include the specular because of the considerable variation in the area of gloss traps used in different spheres. However, the more nearly correct measurements in relation to practical visual observation are with the specular excluded (Best 1987). For computer match prediction of pigmented materials, e.g., paint formulation, the total reflection (i.e., specular included) is preferred. This restriction does not usually apply to tristimulus colorimeters which normally exclude the specular component of reflectance where the illumination viewing geometry is  $45^\circ/0^\circ$ , as is the case in the classic Hunter bench colorimeter.

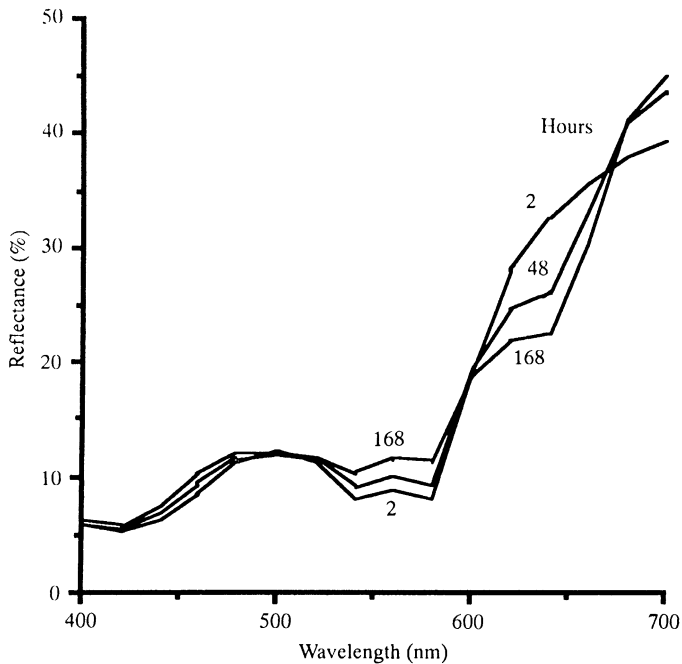
Another important source of variation among tristimulus colorimeters and spectrophotometers is the area of the viewing aperture relative to the area of the illuminating light spot, which affects both the direction and the amount of light returned from translucent materials. MacDougall (1987) demonstrated that translucent suspensions of milk exhibit a tenfold decrease in  $K/S$  for an increase in aperture area from 5 to 20 mm. Best (1987) states that accurate determination of  $K$  and  $S$  by measuring thin layers on black and white backgrounds requires that the ratio of the aperture area to the thickness of the sample must be considerably greater than 10, a criterion unlikely to be met for many foods. One further source of potential error, in addition to those associated with instrument geometry and sample structure, is the wavelength interval used to calculate the tristimulus values. Although the CIE (1986) specifies the standard observer at 5 nm intervals from 380 to 780 nm, such accuracy is not required for most practical purposes. For 10 nm accuracy the intermediate 10 nm values from the 5 nm tables should be used. However, the CIE has not yet officially recommended the use of 20 nm intervals, although many modern colour spectrophotometers detect at 20 nm intervals. Tables of weighting functions at 20 nm intervals for the CIE illuminants and several fluorescent lights are published in the up-to-date colour textbooks cited in this chapter. Errors attributable to wavelength interval are likely to be less important than those from instrument geometry, except when estimating the effects of narrow-band emission lamps on materials with several absorption bands. Here the 20 nm interval may prove to be less efficient.

### 3.9 Food colour appearance measurement in practice

Colour fading from pigment oxidation in fresh meat, the effect of illumination on the appearance of orange juice, the effects of varying coffee and milk concentration on coffee appearance and measuring breakfast cereals by grinding to a defined particle size are given as examples of the types of problems encountered in food colour appearance measurement. The effect of the illuminant on the calculated CIELAB colour values for a variety of food spectra is also presented.

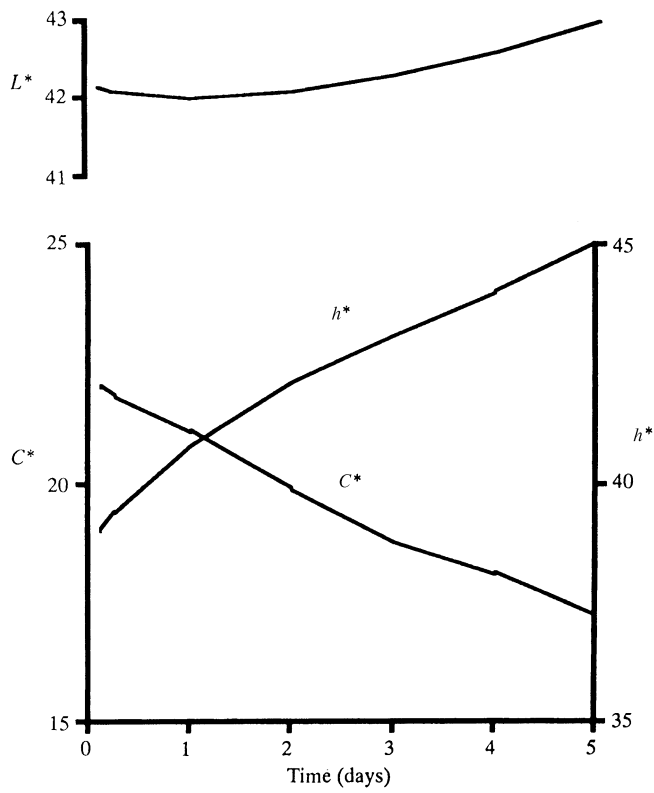
### 3.9.1 Fresh meat

The surface of freshly cut meat oxygenates to bright red on exposure to air from the purple ferrous haem pigment myoglobin to the covalent complex oxymyoglobin. The red oxymyoglobin then oxidises to brownish green metmyoglobin (MacDougall 1982, MacDougall and Powell 1997) during refrigerated display and is affected by both the intensity of illumination and the temperature. Twenty per cent dilution of the surface oxymyoglobin with metmyoglobin causes the product to be rejected at retail because of its faded colour (Hood and Riordan 1973). The changes in the mean reflectance spectra of over 100 packages of beef overwrapped with oxygen permeable film and held in the light at  $< 5^{\circ}\text{C}$  over a period of one week are shown in Fig. 3.5. As the pigment oxidises there is an increase in reflectance in the green region of the spectrum as the alpha and beta absorption bands decrease. This is accompanied with a distinct loss in reflectance in the red region with development of the metmyoglobin absorption band at 630 nm. The changes in colour, calculated for CIELAB for D65, are shown in Fig. 3.6. As meat fades there is a small loss in lightness  $L^*$ , accompanied by much greater changes in  $a^*$  and  $b^*$ . The loss in  $a^*$  and gain in  $b^*$  can be interpreted as an increase in the hue angle  $h^*$  in the direction of yellow with a concomitant loss in chroma  $C^*$ , which is indicative of



**Fig. 3.5** Reflectance spectra of fresh beef during oxidation of oxymyoglobin to metmyoglobin obtained on a diode array spectrophotometer at 20 nm intervals: means of over 100 samples wrapped in oxygen-permeable film and stored at  $< 5^{\circ}\text{C}$  under 1000 lux fluorescent illumination for one week.





**Fig. 3.6** Progressive changes in lightness  $L^*$ , hue angle  $h^*$  and chroma  $C^*$  calculated from spectra of wrapped fresh beef stored at  $<5^{\circ}\text{C}$  under 1000 lux fluorescent illumination during oxidation of surface oxymyoglobin to metmyoglobin.

the colour becoming more grey or dull. The change in direction towards yellow with the reduction in  $C^*$  is perceived as being more brown.

The appearance of meat is greatly affected by the colour-rendering properties of the lamps used for display (Halstead 1978). Some fluorescent lamps recommended by the lamp industry for displaying meat have enhanced red emission which tends to maintain the preferred colour of oxymyoglobin and visually shifts the early stages of metmyoglobin development from brown towards red. This effect of red enhancement on meat colours has been shown to elicit a greater visual colour change in making brown appear red than in making red appear more red (MacDougall and Moncrieff 1988). Some find the flattering of red-enhanced lamps makes meat appear too red.

The ICS Micro Match spectrophotometer used to measure these samples is equipped with the option of using alternative illuminants to calculate CIELAB. The estimated changes in meat colour attributable to different illuminants after one and four days' exposure (Table 3.2) illustrate the effect that light quality has on lightness, hue and chroma. The changes in colour produced by the differences

**Table 3.2** Calculated changes in  $L^*$ ,  $C^*$  and  $h^*$  from D65 to other lamps for the fresh beef spectra shown in Fig. 3.5

Storage time		Difference in colour from D65					
		$\Delta A$	$\Delta WWF$	$\Delta NFL$	$\Delta CWF$	$\Delta 83$	$\Delta 84$
2 hours	$\Delta L^*$	2.9	2.0	0.6	2.0	2.2	1.2
	$\Delta C^*$	7.3	7.1	2.2	7.1	7.6	4.9
	$\Delta h^*$	-2.3	-1.2	3.9	6.5	3.3	-2.3
4 days	$\Delta L^*$	2.3	1.6	0.6	-0.2	1.7	0.9
	$\Delta C^*$	5.7	5.5	2.1	-0.7	6.3	3.8
	$\Delta h^*$	-5.9	-1.9	4.5	9.1	2.8	-3.5

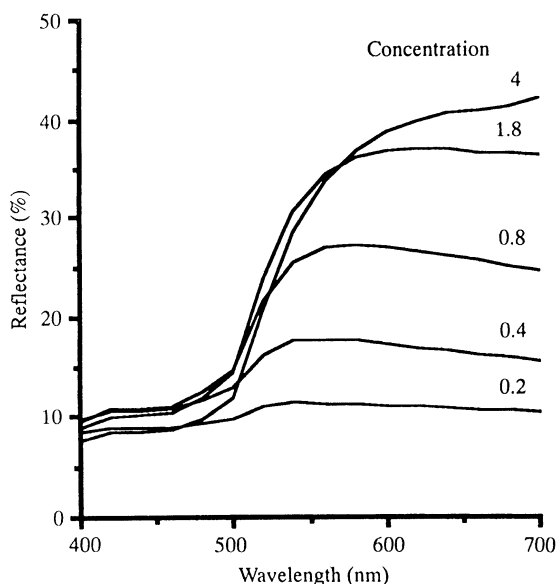
Tungsten lamp: A, fluorescent lamps: WWF, warm white; NFL, natural; CWF, cool white; 83, triband at 3000° K; 84, triband at 4000° K.

in colour rendering among some of the lamps are equivalent to that which occurs after four days' fading, that is  $\Delta L^* \leq 1$ ,  $\Delta C^* \leq -4$  and  $\Delta h^* \leq 7$ . There was little change in  $L^*$  on changing illuminant, but the large changes in  $C^*$  and  $h^*$  illustrate the effects of decreasing lamp colour temperature, altering flattery and improving fidelity. A decrease in colour temperature from D65, as red emission increases, generally increases  $C^*$ , that is the colour is perceived as brighter or more intense with the observer adapted to white. However  $h^*$  may become more brown (more positive) or more red (more negative) as influenced by both colour temperature and the lamp's spectral bandwidth which affects fidelity. This illustrates the potential for mistaken misinterpretation of the data when measuring by the normal procedure of D65 and 10° when, in reality, judgement at retail or in the home will be under warmer illumination.

### 3.9.2 Orange juice

Translucent suspensions are difficult to measure, and direct unobserved interpretation of instrumental data can lead to confusion because of the way the incident light is dispersed in the sample. Most consistent results are obtained if the instrument aperture is large relative to the incident beam (Kent 1987; MacDougall 1987). The effects of optical geometry on colour and the Kubelka-Munk absorption  $K$  ( $\text{mm}^{-1}$ ) and scatter  $S$  ( $\text{mm}^{-1}$ ) coefficients for orange juice were studied by MacDougall (1983) who found  $Y_\infty$ , the luminous reflectance at calculated infinite thickness, increased by 50 per cent if the aperture diameter was increased from 2 cm to 5 cm while the incident beam was maintained at 1 cm.

The effect of dilution of fourfold orange juice concentrate on the reflectance spectra obtained on 4 cm thick samples in thin walled polystyrene bottles is shown in Fig. 3.7. As can be seen, 4 cm is practically equivalent to infinite thickness. Kubelka-Munk absorption and scatter coefficients were calculated from 2 mm thick samples on black and white backgrounds. On dilution,  $S$

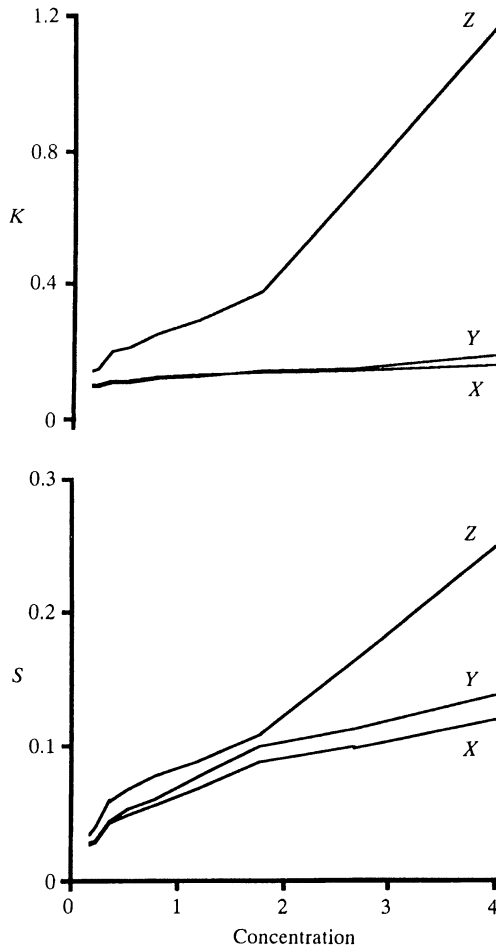


**Fig. 3.7** Reflectance spectra of concentration and diluted orange juice at a path length of 4 cm, equal to infinite thickness: reconstituted juice at normal concentration = 1.0.

decreased for  $X$ ,  $Y$  and  $Z$  as the suspension became more translucent.  $K$  for  $Z$  decreased to approach the much lower near constant values of  $K$  for  $X$  and  $Y$  (Fig. 3.8). This decrease in  $K$  for  $Z$  is as anticipated for a blue absorbing pigment. The effect of loss of scattering power on dilution was to reduce  $Y_{\infty}$ , and hence lower  $L^*$ . The most dilute juice, therefore, is instrumentally the darkest, and the most concentrated is the lightest (Fig. 3.9). However, this is not what is perceived. Glasses of orange juice viewed with overhead illumination range from pale yellow for concentrations less than 1 to deep orange at a concentration of 4, which is opposite to that determined instrumentally. For strongly scattering coloured materials in dilute suspension, measured colour, even supplemented by information on scatter, is inadequate to fully describe appearance. The instrument does not measure what the observer sees because light is reflected from a limited solid angle, whereas the observer's perception is influenced by the multidirectionality of illumination, which makes coloured translucent materials appear to glow.

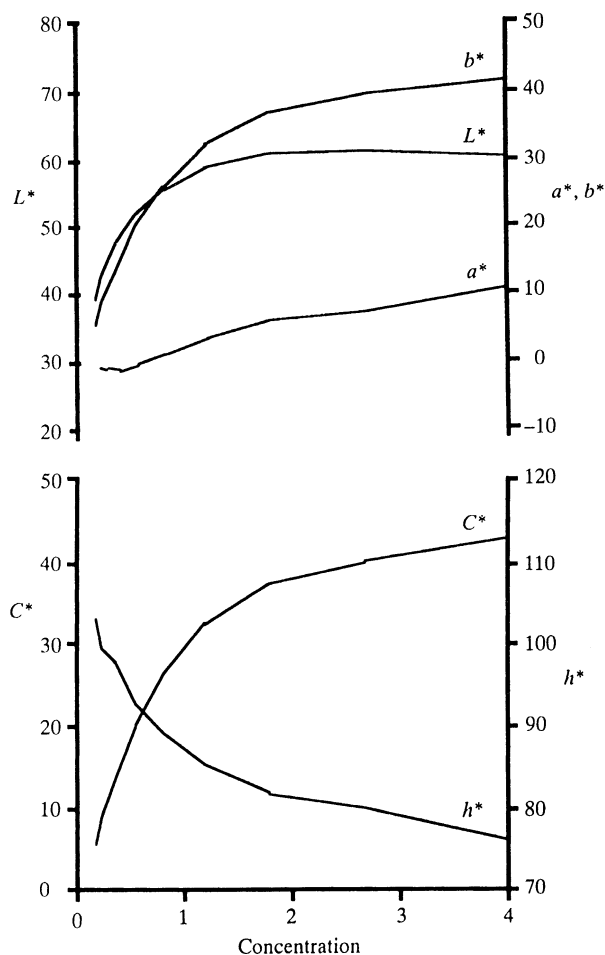
### 3.9.3 Coffee

Coffee in the cup is usually poured black for the drinker to adjust its colour by adding milk or cream. The drinker uses the combination of milk and coffee as an indication of its anticipated taste although he or she may not know the actual concentration of coffee in the drink. The most obvious appearance attribute of milk in coffee is the intensity of its colour, but translucency has also been shown to affect visual estimation of coffee strength (Hutchings 1999; Mackinney and



**Fig. 3.8** Kubelka-Munk absorption  $K$  and scatter  $S$  coefficients for tristimulus values  $X$ ,  $Y$  and  $Z$  for concentrated and diluted orange juice: values ( $\text{mm}^{-1}$ ) calculated from reflectance spectra obtained from 2 mm path length cells with black and white backgrounds.

Little 1962). The Kubelka and Munk theory of mixing pigments in a light-scattering suspension (Judd and Wyszecki 1975; Kubelka 1948) is used as the basis for colour formulation in the paint, plastics and textile industries (Nobbs J. 1997; McDonald R. 1997). By manipulating the relationship of the concentration of pigment ( $K$ ) to degree of light scatter ( $S$ ) it was demonstrated that it is possible to produce approximately equivalent levels of visual lightness in up to fivefold dilutions of brightly coloured milk (MacDougall 1988). For coffee, since the colour is dull brown, then neither the lightness, chroma nor hue, from different concentrations of the components would be expected to change markedly for the drink viewed in the cup, provided the  $K/S$  ratio remains constant and the drink does not become obviously translucent.

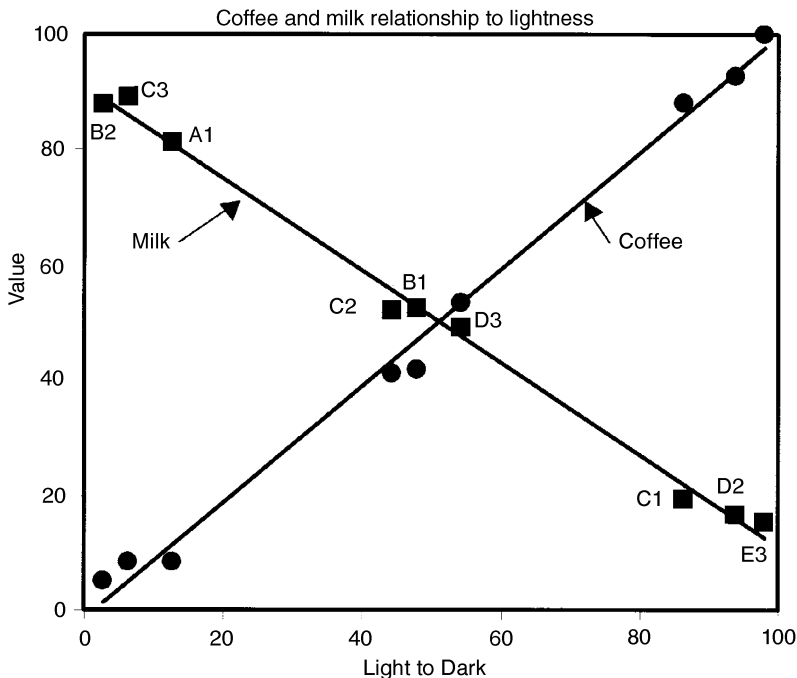


**Fig. 3.9** Changes in lightness  $L^*$ , opponent co-ordinates  $a^*$  and  $b^*$ , hue angle  $h^*$  and chroma  $C^*$  calculated from reflectance spectra of concentrated and diluted orange juice at 4 cm path length.

Hutchings (1999) previously had observed that the appearance of creaminess and coffee concentration was related to the  $K/S$  ratio but his studies did not include assessment of the actual drinking quality of the beverage. MacDougall and Lima (2001) recently studied the relation of the appearance of coffee at a range of constant  $K/S$  values to apparent coffee concentration and milk concentration coupled with a sensory panel's detection of the strength of the components in the drink. The three ' $K/S$  constant ratios' of coffee to milk were made from three levels of Nescafé Gold Blend instant coffee and pasteurised semi-skimmed milk, i.e., nine mixtures in all. Unit quantity of coffee was defined as 2.5 g/l and unit quantity of milk as 100 ml/l. The constant ratios were constructed by doubling the quantity of both coffee and milk at each level and

the differences between the ratios by doubling the concentration of coffee from the previous ratio. This required three levels of milk, 100, 200, and 400 ml/l, designated 1, 2 and 3, and five levels of coffee, 2.5, 5, 10, 20 and 40 g/l, designated A, B, C, D and E. Not all combinations were required for the three constant ratios of (A1, B2, C3), (B1, C2, D3) and (C1, D2, E3), i.e., coffee to milk ratios of 1:1, 2:1 and 4:1. It is to be noted that 2.5 g/l of freeze-dried coffee is equal to one heaped teaspoonful, a quantity typically used in making a cup of coffee.

Samples of the cold coffee mixtures were presented in 1.5 cm deep tissue culture bottles, which have a 5 cm<sup>2</sup> clear face, illuminated by Artificial Daylight Fluorescent Lamps (D<sub>65</sub>) at 900 lux. Twenty assessors, with normal colour vision, spaced the nine bottles on three 150 cm unmarked straight edges on a pale grey bench for degree of lightness to darkness, for coffee strength and of milk strength relative to the most extreme samples located at the respective ends of the scales. The mean spacing of the samples is given in Fig. 3.10 where they were judged to cluster according to the three constant *K/S* ratios even though the concentration of milk and coffee in the most concentrated sample within each ratio was fourfold higher than the lowest. The differences within the constant ratios were much less than between the ratios showing that the effect of adding milk produced considerable confusion in the observers' ability to identify the



**Fig. 3.10** Relationship of visual lightness to darkness of coffee and milk *K/S* mixtures to the perception of the strength of the coffee and milk.

concentrations of the components. That is, up to a fourfold increase in pigment absorption is apparently nullified by a similar increase in light scatter. When the sensory panel observed and tasted the same samples, this time prepared hot, they similarly separated their appearance when presented in white cups. The taste attributes of coffee and milk were more complexly related to their appearance. Quantitative descriptive analysis of the panel data separated the attributes primarily on the constant  $K/S$  ratios but interactively on the absolute values of their components. This demonstrates that the visual appearance of coffee and milk is not a reliable indicator of the taste.

### 3.9.4 Breakfast cereals

Discontinuity in breakfast cereals may be defined as that condition where the components of the product and the inter-component spaces are arranged randomly, which does not lend itself to easy and reproducible measurement. The problem would appear to be intractable unless samples are manipulated to replace the discontinuity with a uniform distribution of the particles. One approach is to grind the sample to constant particle size (Kent 1987). This author studied the colour of four UK breakfast cereals (Shredded Wheat, Weetabix biscuits, Kellogg's Corn Flakes and Bran Flakes) ground to >2mm, 1–2mm, 0.5–1mm and <0.5mm through BSI standard sieves. The increased surface area as the particles reduced in volume scattered more light but removed randomness. The 1–2mm and 0.5–1mm fractions were closest in appearance to the original flakes, and to each other, whereas the <0.5mm fraction in each case was considerably lighter and more yellow. In addition to measuring the cereals' colours, reference colours were used to visually define the degree of discrepancy between measurement and visual observation of the material (MacDougall 2001). This was accomplished with NCS colour patches under both Artificial Daylight ( $D_{65}$ ) and tungsten illumination. The greatest differences in  $L^*$ ,  $C^*$  and  $h^*$  between 1–2mm samples for all cereals and their nearest colour patch were in  $L^*$  which ranged from 7 to 21 units and always in the same direction of the NCS colours being the lighter. Differences in  $C^*$  were <3 units and  $h^*$  were <7 degrees except for bran flakes which was 15 degrees because of difficulty in deciding which atlas page most resembled the product. The greatest contribution to  $\Delta E^*$  therefore, was  $\Delta L^*$ , with chroma and hue virtually unchanged. Typical differences in  $L^*$  between adjacent colours in the atlas are approximately 10 units. This demonstrates that the magnitude of visual discrepancy in colour assessment, i.e., the error, of products prepared in this fashion will be greater than differences between adjacent NCS patches.

## 3.10 Illuminant spectra and uniform colour

Although  $D_{65}$  is the reference illuminant spectrum most used for calculating CIELAB, of the other lamps listed in Table 3.2 the more important for relating

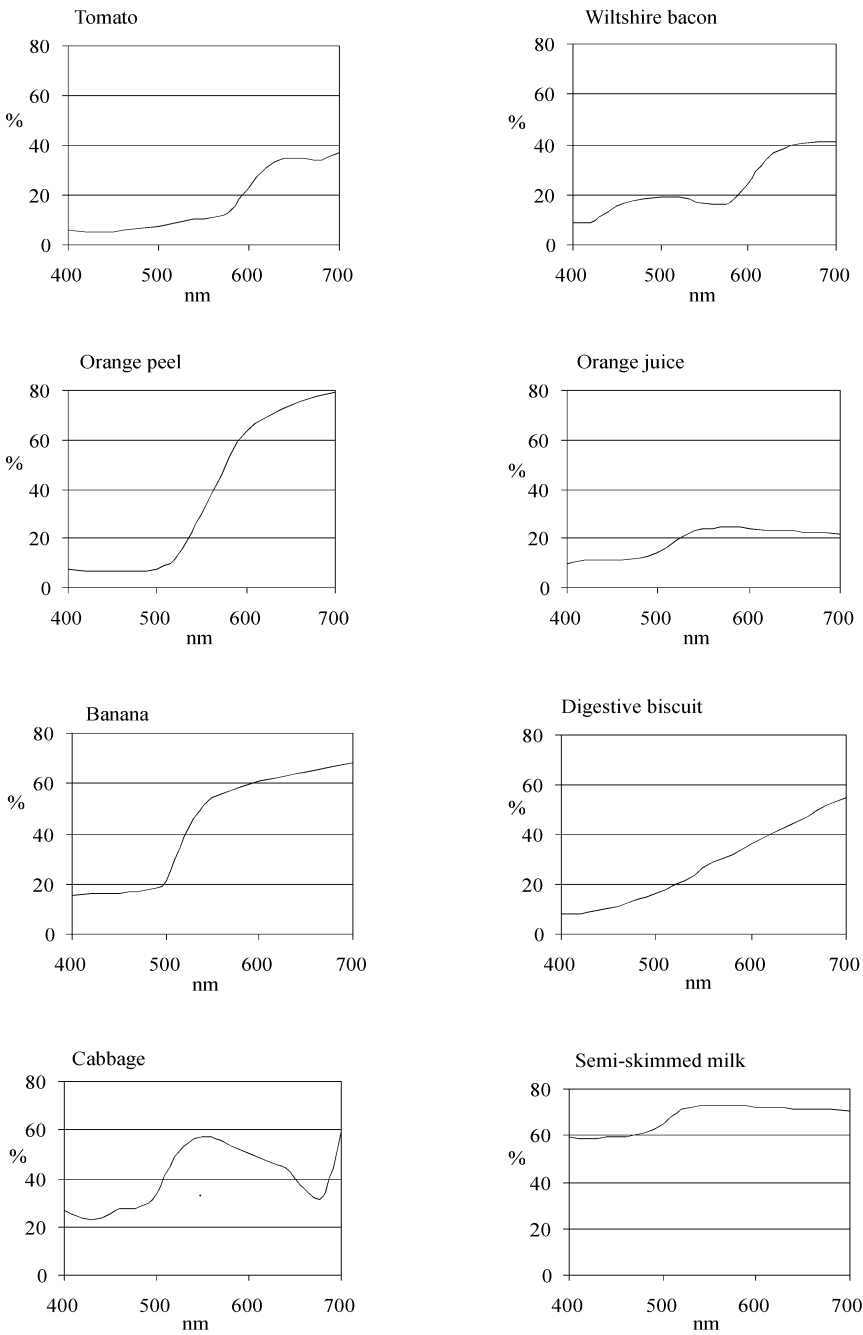
food colour measurement to visual colour in practice are A and 83. A is the emission spectrum of tungsten illumination and 83 is that of 'tri-band' fluorescent illumination, similar to F11 in Fig. 3.3. However in 83 the red phosphor is increased by >25 per cent over that of F11 and the green phosphor reduced by about 12 per cent giving the lamp a warm appearance (3000°K) similar to that of tungsten but with a considerable reduction in energy utilisation. 83 is now commonly used for food display in supermarkets because of its attractiveness, pleasant and realistic colour rendering and low heat output. To examine the size of the effect of these lamps on CIELAB colour space, a variety of foods, selected as representative of the major hues, were measured on the ICS Micro Match spectrophotometer. The red hue examples were fresh tomatoes at supermarket readiness, i.e., not excessively ripe but bright attractive red-pink and Wiltshire bacon, typically translucent in appearance. The yellow spectrum was the mean of several bananas, ripe but without any indication of brown spotting. The difference between the two orange samples was that the colour of the peel of entire navel oranges is near opaque and brilliant, whereas the colour of freshly squeezed orange juice is translucent. This results in a large reduction in the juice's reflectance at the red end of the spectrum compared to that of the peel. The green spectrum was that of the external leaves of a mid- to light-green cabbage and the brown was that of the surface of a 'digestive' biscuit. The near-white spectrum was that of semi-skimmed milk. All samples were measured at infinite thickness.

Figure 3.11 shows the average reflectance spectra of the foods and Fig. 3.12 the location of the samples in CIELAB calculated for the three illuminants. The triplets of points for each sample show that D65 has lower  $b^*$  values, that is more blue as would be expected, and A and 83 are more yellow with A more red than 83 with higher  $a^*$  values. These results parallel visual observation of the products viewed under tungsten, artificial daylight (D65) and Philips 83 lamps. This study clearly demonstrates that if interpretation of CIELAB is to be related to illumination conditions in practice, then D65 should not be the only illuminant used to calculate the colour of the food.

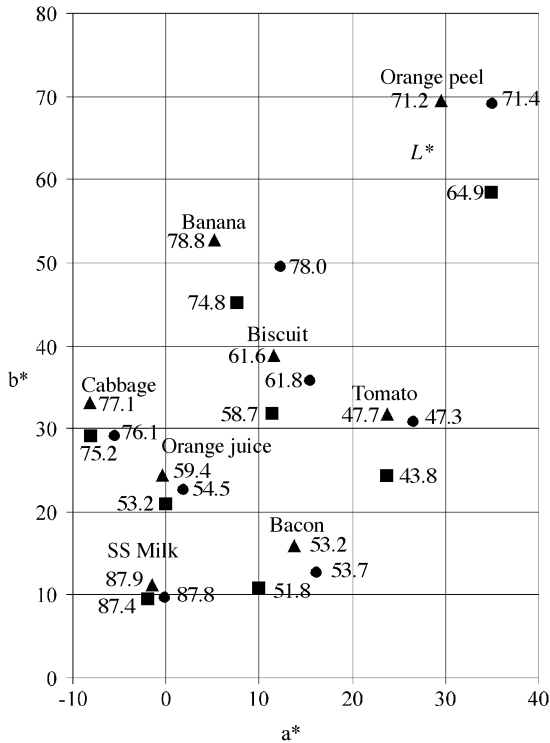
### 3.11 Conclusions and future trends

This chapter has attempted to present the basics of colour measurement as applied to foods. It is important to realise that the wide variability in the nature of foods and food products, from both their structure and pigmentation, may limit any colour measurement technique applicable only to that particular food. This is because the numeracy of the data is unlikely to match the visual experience of equivalent reference atlas colours. In some cases this discrepancy may be large and has to be recognised as an intrinsic property of the food. These differences may arise from the difference of the visual experience of the product when viewed under normal lighting conditions as opposed to the limitations of its optical properties when presented to the particular colour-measuring instrument. This is





**Fig. 3.11** Reflectance (%) spectra of a selection of foods of differing hue.



**Fig. 3.12** CIELAB  $a^*b^*$  spacing of the food spectra from Fig. 3.11 calculated for the illuminants D65 (■), A (●) and 83 (▲). The  $L^*$  values for each food are given adjacent to the symbols of the illuminants.

particularly so in the case of translucent foods. The values of measured lightness, hue and chroma are likely to be quite different from similarly coloured opaque materials. A consequence of this is that, if required for quality decisions, the variability of measured colour for any food over the range of acceptability and unacceptability needs to be established relative to real samples judged under appropriate controlled visual judgement conditions. In the light of recent studies on the increased accuracy of colour difference and perceptual acceptability formulae the industry may have to reassess its procedures. Whether or not the level of accuracy demanded by other colour industries is required by the food sector remains to be proved. If the foregoing are taken account of the use of colorimetry is likely to expand in the food industry in the future, especially so as portable instruments and in-line measurement techniques become accepted as reliable indicators of product quality. Care needs to be exercised in using such instruments because their detector geometries are different from that of larger reference spectrophotometers.

On-line use of colour measurement is likely to increase especially where the colour values obtained by the instrument can be incorporated automatically into

process control. The use of video image analysis (VIA) to couple colour measurements with variation in appearance is likely to increase. Provided the RGB signals from VIA systems and digital camera output can be translated into meaningful colour data then a wider and more adaptable approach to food colour appearance control might be possible. VIA systems could become a more direct way of relating measurement to visual assessment and effectual quality assurance. Such systems in conjunction with standard colour measurement could possibly provide direct quality links by computer from the supplier to the processor to the supermarket

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## 4

# Models of colour perception and colour appearance

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### 4.1 Introduction: colour specification systems and colour appearance models

Over the last decade it has become clear that a model that can predict the colour appearance of surfaces in complex images is required by practitioners in manufacturing industries including food production. Such a model should be able to predict colour constancy and simultaneous contrast. However, colour appearance is a complicated phenomenon that is still not completely understood and ultimately the challenge is to develop colour-appearance models that can predict contrast and induction effects as well as other documented phenomena such as the Helson-Judd effect (the tendency of lighter achromatic surfaces to take on the hue of the illuminant under which they are viewed and darker achromatic surfaces to take on the complementary hue) and the Helmholtz-Kohlrausch effect (the increase of brightness, or lightness, of colours having higher chroma or colourfulness).

The original CIE system of colorimetry was designed for colour specification and has only limited use as a colour-appearance model. It can be easily shown that the CIE tristimulus values that can be used effectively to specify a surface colour do not correlate well with the appearance of that surface. This chapter introduces the distinction between colour specification and colour appearance and then reviews how simple adaptive processes that pool information at different spatial positions in an image can be used as a basis for colour-appearance models. A typical colour-appearance model, CIECAM97s, is then described. Finally, some further uses of colour-appearance models are discussed.

## 4.2 The retinal image

### 4.2.1 Colour appearance and colour specification

The retina contains three classes of cells, known as  $L$ ,  $M$  and  $S$  cones, that are responsible for colour vision and that are maximally sensitive to long-, medium-, and short-wavelength light in the visible spectrum. The relationship between the spectral reflectance  $R$  of a surface and the excitations  $e_i$  (where  $i \in \{L, M, S\}$ ) of the cones that occur when the surface is viewed in a light source defined by a spectral power distribution  $E$  is often expressed by the equation

$$e_i = \sum_{\lambda} R(\lambda) E(\lambda) \Phi_i(\lambda) \quad 4.1$$

where  $\lambda$  represents wavelength and  $\Phi_i$  are the cone fundamentals. The cone fundamentals provide the effective spectral sensitivities of the cone classes and include the spectral sensitivities of the cone pigments themselves in addition to other processes such as the absorption of short-wavelength light by the yellow lens and by the presence of macula pigment in the retina. The cone excitations computed according to Equation 4.1 specify the colour stimulus that is defined by the combination of the surface  $R$  and the illuminant  $E$ . An equivalent specification can be computed by replacing the cone fundamentals in Equation 4.1 by the CIE colour-matching functions to yield the CIE XYZ tristimulus values. Such colour specification is useful since it defines the conditions under which, for example, two surfaces with differing reflectance properties will be equivalent and hence match. The equivalence of the colour specification is conditional if the reflectance properties of two surfaces are different and cannot be guaranteed if the light source is changed or if the observer has properties different from those as specified by the standard cone fundamentals or the CIE colour-matching functions. However, the fact that two colour stimuli match according to Equation 4.1 does not provide any information about the actual appearance of the two surfaces.

The difference between colour specification and colour appearance can be highlighted if we consider the everyday phenomenon of colour constancy. Given Equation 4.1 it can easily be demonstrated that the triplet of cone excitations for a given surface varies considerably when the surface is considered to be viewed under different illuminants. (The CIE XYZ tristimulus values for a surface can similarly be shown to vary with the illuminant.) Nevertheless, when viewed under a wide range of light sources most surfaces approximately retain their colour appearance despite the changes in the cone excitations that are suggested by Equation 4.1. This phenomenon of colour constancy has been known at least since Helmholtz (see Worthey, 1995). In the official terminology of the CIE we can say that a change of the illuminant on a scene imposes a colorimetric shift on each object in the scene. Thus neither the cone excitations computed according to Equation 4.1, nor the XYZ values, can accurately predict the colour appearance of objects. In fact, the colour appearance of the object is predicted better by the object's surface-reflectance properties than by the spectral distribution of the light reflected by the surface or by the CIE tristimulus values.



It is important to understand that the results from colour-matching experiments, such as those used to determine the CIE colour-matching functions, can be used to predict when two stimuli will look the same in certain circumstances, but cannot be used alone to tell us what the stimuli will look like (Hurlbert, 1991).

#### 4.2.2 Spatial properties of the retinal image

One of the limitations of the original CIE system of colorimetry is that a colour stimulus is considered in spatial isolation whereas evidence will be presented in this chapter to show that colour vision is inseparable from spatial vision. Whereas Equation 4.1 encapsulates the main colour properties of the cells in the retina we need also to be aware of the spatial properties of retinal processing. For example, it can be considered that at the level of the retina the visual system captures three separate images of scenes that are viewed, one image for each of the cone classes. There are differences, however, in the spatial properties of the cells between the cone classes. The *L* and *M* cones are very much more numerous than the *S* cones. Indeed, it has been demonstrated (Williams *et al.*, 1981) that at the centre of the retina (the fovea) there are no *S* cones whatsoever (this physiological property leads to the phenomenon of small-field dichromacy whereby observers can match small visual stimuli using just two primaries rather than the usual three). Even at greater retinal eccentricities there are very many fewer *S* cones than either *L* or *M* cones and consequently the retinal spacing between the *S* cones is relatively large. The *L* and *M* cones therefore capture an image of the scene at a higher spatial resolution than the *S* cones are capable of.

#### 4.2.3 Chromatic aberration

Light that enters the eye is focused onto the retina by means of the lens. The change in refractive index that occurs when light moves from air to the glass-like structure of the lens causes the rays to be refracted. The shape of the lens can be modified by the ciliary muscles and thus the process of accommodation provides the correct amount of refraction to give the appropriate lens power. However, the amount of refraction that occurs is a function of wavelength and therefore the visual system can focus perfectly on only one wavelength at any given time. It has been shown that the eye will naturally focus on a wavelength of about 580 nm (a wavelength that is quite close to the wavelengths of maximum sensitivities of the *L* and *M* cones) and therefore light at shorter and longer wavelengths in the visible spectrum is not in perfect focus (Marimont and Wandell, 1993). This phenomenon is known as chromatic aberration. The wide spacing of the *S* cones and the effect of chromatic aberration of the lens can therefore be seen to be complementary. Even if the *S* cones were more closely packed it would not be possible to obtain a 'blue' image of high spatial resolution because of the deteriorating effect of chromatic aberration on the spatial properties of the short-wavelength image.

#### 4.2.4 Spatiochromatic properties of the visual system

The colour sensitivity of the cone classes, the physical spacing of the cones within the retina, and the effects of chromatic aberration at the lens all contribute to what we call the spatiochromatic properties of the visual system. Whereas the CIE colour-matching functions describe the colour properties of the visual system reasonably adequately, if we wish to be able to analyse the colour-appearance properties of colour images or scenes then we need models that can incorporate the spatiochromatic properties of the visual system. In the next section some colour-appearance phenomena are described that demonstrate the intrinsic link between colour and spatial properties.

### 4.3 Colour appearance: colour constancy

The phenomenon of colour constancy cannot easily be explained by a model of the visual system based only upon Equation 4.1 (and therefore, equivalently, by the CIE 1931 and 1964 CIE systems) because surfaces retain their approximate colour appearance when the illumination is changed even though the cone responses defined by Equation 4.1 change with the illumination. Nevertheless, within a uniformly illuminated image, bright objects consistently generate more cone absorptions than dark objects; red objects generate more *L*-cone absorptions than either blue or green objects, and blue objects generate more *S*-cone absorptions. It would thus appear that spatially relative cone excitations might predict colour appearance better than the local cone excitations themselves. The colour seen in a region of space is determined not only by the characteristics of the stimulus in that region, but also by those simultaneously present in surrounding regions. Important experimental data are provided from a class of techniques generally referred to as asymmetric colour-matching experiments. In asymmetric experiments, unlike in conventional colour matching, matches are determined between stimuli presented in different contexts. In memory-matching versions of the experiment, for example, the observer studies the colour of a surface presented under one illumination source and then must select a target that looks the same under a second illumination source. These measurements identify the stimuli, and thus the cone absorptions of those stimuli, that look the same under the two illuminants.

The results from early colour-matching experiments of this type were explained by von Kries (Von Kries, 1904) who suggested that the visual pathways adjust to the illumination by scaling the signals from the individual cone classes. Von Kries scaling predicts that the cone absorptions for stimuli that match under two illuminants (such stimuli are also known as corresponding colours) should be linearly related. An analysis of some experimental data from Wassef (1959) by Wandell (1995) illustrates that whereas simple von Kries scaling cannot exactly predict the cone absorptions of corresponding colours it is a good first-order approximation of the phenomenon. The von Kries scaling law can be written as

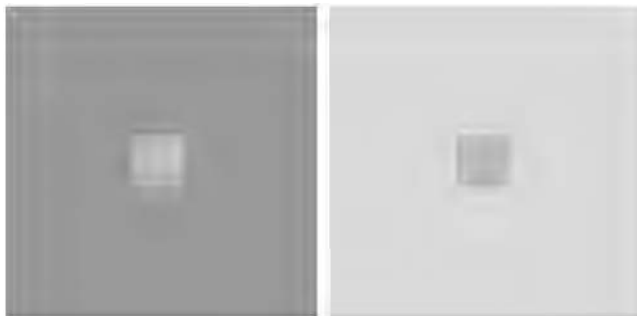
$$\Phi'_i = k_i \Phi_i \quad 4.2$$

where  $\Phi_i$  represents the cone absorptions under the first illuminant (where  $i \in \{L, M, S\}$  as before) and  $\Phi'_i$  represents the cone absorptions under the second illuminant. These latter cone absorptions are sometimes called the adapted responses or integrated reflectances (McCann *et al.*, 1976).

The von Kries scaling law suggests that either the main mechanism that accounts for colour constancy occurs early in the visual processing or else that the photoreceptor signals can be recovered and are available during cortical processing. Note that the von Kries rule assumes that the shapes of the spectral-sensitivity functions of the three classes of photoreceptors stay the same but that their overall sensitivities adapt to the properties of the scene being viewed. Although the shapes of the spectral sensitivity functions for the cone classes remain constant, a likely implementation of von Kries scaling can occur physiologically simply as a process of adaptation. There is ample evidence from psychophysical, physiological and spectrophotometric studies of retinal function to support the existence of photochemical bleaching (De Valois and De Valois, 1975). That such adaptation processes take place in the retina is fairly well established and these mechanisms allow the visual system to be responsive to a wide range of brightness values.

#### 4.4 Colour appearance: simultaneous colour contrast

A further limitation of the original CIE system is illustrated by Fig. 4.1 where the two grey patches are physically identical, create the same local rate of photopigment absorption, and give the same cone excitations according to Equation 4.1. Yet, they appear to be very different in lightness because of the difference in their excitations relative to nearby areas. Thus whereas colour constancy demonstrates that patches of colour that have different tristimulus definitions can have the same colour appearance, simultaneous colour contrast demonstrates that patches of colour that have the same tristimulus definitions



**Fig. 4.1** The two small grey squares are physically identical and yet their appearances are quite different

can have different colour appearance. Furthermore, this shows not only that cone responses are influenced by the illumination in the scene but also that they spatially interact with each other. Thus, colour vision and spatial vision are inseparable.

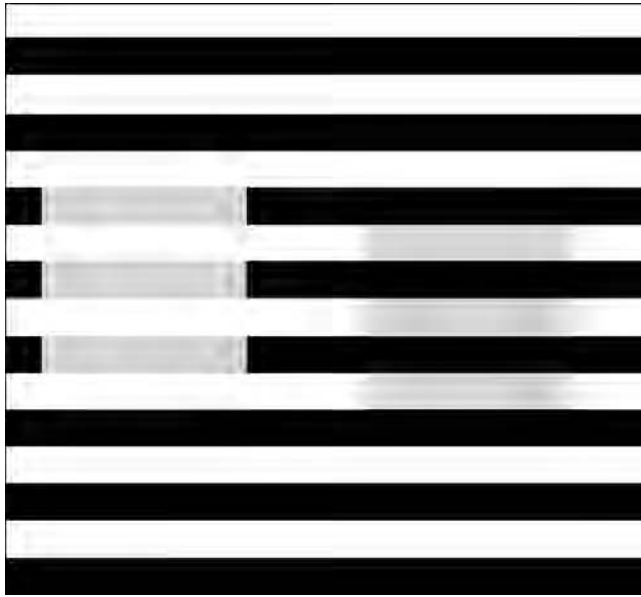
Contrast effects are well known and have been extensively investigated. Although it is widely known that a grey square looks brighter on a black background than it does on a white background the influence of contrast effects on everyday vision is less well appreciated. Thus, a piece of coal in bright sunlight may reflect thousands of times the amount of light (per unit area) to the eye as does a white piece of paper in an adjacent shadowed area. Despite this the coal is seen as black and the paper is seen as white. Whether we see an object as white, grey, or black is almost totally determined by the relative amounts of light it and its surround reflect; it is a function of the contrast rather than of the intensity of the light (De Valois and De Valois, 1975). The physiological mechanism for such effects certainly involves processes of lateral inhibition. Retinal ganglion cells in the retina are known to have receptive fields that consist of two concentric areas; a central excitatory area and a surrounding inhibitory opponent area (Kuffler, 1953). These cells are called on-off cells but off-on varieties are known to exist too where the central area is inhibitory.

A more complex appearance phenomenon occurs when observers stare fixedly at a colour stimulus for a length of time and then view a uniform field of different colour. Thus if a red square is viewed on a grey background and then replaced by a uniform grey field the observer will see a green square where the red square was. Helmholtz (1866) offered a physiological explanation for such successive colour contrast or negative after-images. His explanation of the example of looking at a grey surface after prolonged inspection of a red square was that the inspection of the red square would lead to bleaching of the *L*-cone pigment. However, it is now known that powerful after-images can be perceived at low light levels where little pigment bleaching could have occurred (De Valois and De Valois, 1975). Also, after an exposure to a red stimulus a green after-image can be perceived even in the dark, which refutes the excitation of the *S* and *M* cones more than the *L* cones as a complete explanation.

A further physiological process is required to explain colour appearance during successive colour contrast. A ubiquitous property of neurones almost anywhere in the cortex is that when prolonged stimulation is terminated the firing rate of the neurones rebounds in the opposite state (De Valois and Jones, 1961). Since the visual pathways carry information in white-black or colour opponency channels, the termination of a stimulus will lead to negative after-images even in the absence of any further receptor stimulation.

## 4.5 Colour appearance: colour assimilation

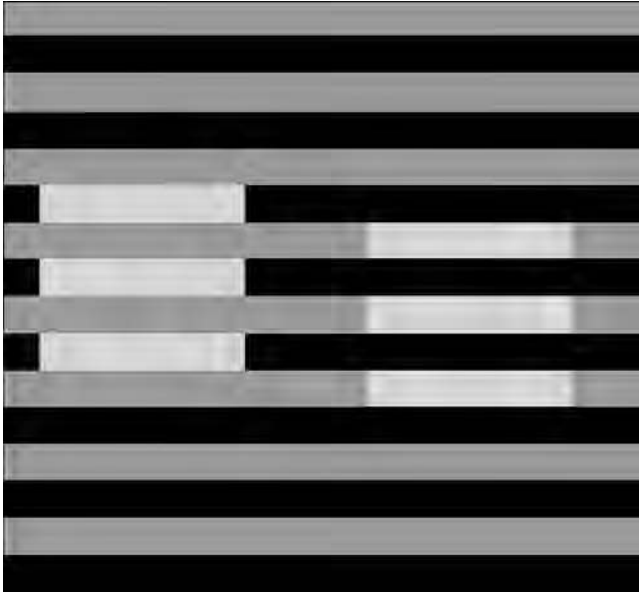
Simultaneous colour contrast is usually considered to be the case where the colour of an area is changed towards the opposite colour of the surrounding



**Fig. 4.2** (a) White's Effect. The small grey patches are all physically identical. The patches on the left might be expected to appear darker than those on the right because they are mainly surrounded by white. However, the opposite shift in colour appearance occurs (White, 1979).

patch relative to when the area is seen in isolation. However, the opposite effect can also occur. These anti-contrast changes have been called the von Bezold effect and are also known as assimilation. An interesting example of assimilation is known as White's effect (White, 1979) and is demonstrated in Fig. 4.2a.

In Fig. 4.2a the grey patches on the left of the figure appear lighter than those on the right. Simple contrast effects, however, would predict the opposite since the grey patches on the left are mainly surrounded by white horizontal bars and those on the right are mainly surrounded by black horizontal bars. The appearance of Fig. 4.2a has been explained by the synergy of two factors: assimilation with the top stripe and contrast with the interrupted stripe (Ripamonti and Gerbino, 2001). Even more interestingly, standard contrast effects are observed in Fig. 4.2b; Ripamonti and Gerbino (2001) refer to the inverted White's effect when discussing this figure. Notice that in Fig. 4.2a the luminances of the patches are between the luminances of the horizontal stripes whereas in Fig. 4.2b this is not the case (Spehar *et al.*, 1997). The complexity of these explanations is not encouraging for researchers who would like to develop colour-appearance models since they could be interpreted as requiring a top-down approach to vision whereas most practical models are designed to be bottom-up.



**Fig. 4.2** (b) The induction effects that are seen in Fig. 4.2a do not occur in Fig. 4.2b where the luminances of the smaller patches are no longer between the luminances of the main horizontal stripes. Ripamonti and Gerbino (2001) refer to this as Inverted White's Effect.

## 4.6 The nature of colour contrast

The retinal neurons have an individual response range of two or three orders of magnitude and yet must remain sensitive to image patterns despite the fact that the ambient light intensity can vary over six orders of magnitude from a dim evening to a sunny day. One solution to this problem is achieved by coding the local contrast in the image rather than the absolute stimulus level. The range of contrasts in a typical image remains almost constant as the ambient illumination level changes. By coding contrast, neurons with a relatively small dynamic range can convey important information about the retinal image despite the large variations in the absolute levels of light. The sensitivity to contrast is therefore an important and useful measure of the spatiochromatic properties of the visual system.

### 4.6.1 What is contrast?

The usual definition of the contrast  $Ct$  of a stimulus is given by the Michaelson contrast equation

$$Ct = (I_{max} - I_{min}) / (I_{max} + I_{min}) \quad 4.3$$

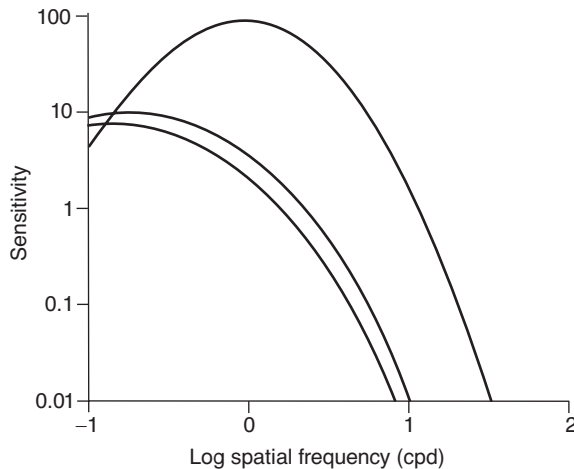
where  $I_{max}$  and  $I_{min}$  are the maximum and minimum intensities in the stimulus. Imagine an image that shows an achromatic horizontal sinewave grating that

varies from white at  $80 \text{ cd/m}^2$  through to black at  $20 \text{ cd/m}^2$ . The average contrast of the stimulus is  $50 \text{ cd/m}^2$  and the Michaelson contrast is 0.6. The maximum contrast possible with this stimulus would occur if the maximum and minimum intensities were set to  $100 \text{ cd/m}^2$  and  $0 \text{ cd/m}^2$  respectively and in this case the Michaelson contrast would be 1.

#### 4.6.2 Contrast sensitivity functions

Contrast sensitivity is normally assessed by displaying sinewave gratings of specific spatial frequency and measuring the amount of contrast that is required so that the observer can just detect the presence of the grating against a uniform field of matched average luminance. This is therefore a threshold detection experiment and sensitivity can be defined as the inverse of the contrast at the threshold. A plot of contrast sensitivity as a function of spatial frequency is called the contrast sensitivity function (Campbell and Green, 1966) and this is usually measured for achromatic stimuli (the luminance channel) and for two classes of iso-luminant stimuli (the red-green channel and the yellow-blue channel). These three colour directions are selected because they represent the opponent colour channels that are believed to be used by the visual system to encode the cone responses. The contrast sensitivity function has been measured by a number of researchers and is now well established (Noorlander and Koenderink, 1983; Mullen, 1985). Figure 4.3 shows the contrast sensitivity functions for these three cardinal colour directions and several properties can be observed.

Firstly, it can be seen that the sensitivity of the luminance channel is much higher than the sensitivities of the two iso-luminant opponent channels. Secondly, the upper spatial frequency to which the channel still remains



**Fig. 4.3** Schematic diagram to show the contrast sensitivity functions of the luminance, red-green and yellow-blue colour directions.

sensitive is much higher in the luminance direction (about 60 cycles/deg) than in either of the other two directions (this shows that the spatial acuity of the luminance channel is much higher than that of the other opponent channels). Thirdly, the shape of the luminance contrast sensitivity function is different from those of the colour channels. The luminance channel peaks at about 6 cycles/deg and is described as being band-pass whereas the chromatic channels are low-pass since their responses consistently decrease with increasing spatial frequency. Note that this implies that whereas the chromatic channels have maximal sensitivity for uniform fields, this is not the case for the luminance channel which is more sensitive to patterns with a spatial frequency of about 6 cycles/deg than to uniform fields. The luminance channel therefore responds best to spatial contrast and plays an important role in human vision in terms of edge detection and form detection in general.

The information presented in Fig. 4.3 does not tell the whole story about contrast sensitivity. For example, the shape of the luminance contrast sensitivity function changes with the luminance level and becomes more low-pass at low levels of average luminance. Furthermore, there is the issue of how the contrast sensitivity of a complex stimulus can be predicted from the contrast sensitivity measurements taken from simple stimuli. Complex stimuli are stimuli containing more than one spatial frequency and natural images, and images taken of foodstuffs, are therefore complex images. Campbell and Robson (1968) measured how well observers can discriminate between a square wave (consisting of many spatial frequencies) and a sine wave at a single spatial frequency. The two stimuli (which have the same fundamental frequency) can be discriminated by the contrast of the higher frequencies. If discrimination occurs at contrast levels at which the higher frequency alone would not normally be visible, then we say that facilitation has taken place. Thus, the higher spatial frequency is easier to detect in the presence of the lower spatial frequency. Similarly, if discrimination requires a contrast in excess of that required for the detection of the higher frequency, then we say that masking has occurred. Facilitation and masking have been frequently found in experimental data (Legge and Foley, 1980) although surprisingly the results from Campbell and Robson (1968) did not exhibit either (Wandell, 1995).

## 4.7 Modelling colour appearance

It has been shown that colour appearance is a complex phenomenon and a deep understanding of the properties and function of the human visual system is required in order to understand it fully. A model of the human visual system is probably many decades from fruition, if indeed it is possible at all. Despite this, some of the properties of the visual system that have been discussed have been understood and implemented in models that can predict the quality of images, the visual difference between two images, and colour appearance of simple and complex images. An exhaustive list of these models is not presented here.



Rather, some typical models are briefly described in terms of their function and the way in which they exploit known properties of the visual system.

#### 4.7.1 Image-quality assessment

Several models exist for comparing one image with another or for the assessment of the quality of a digital image. Metrics such as S-CIELAB (Zhang and Wandell, 1997) and Daly's visible-difference prediction model (Daly, 1993) are used to quantify differences between an original image and its reproduction in terms of the human visual system. S-CIELAB converts the image information into three separate image planes expressed in terms of luminance, red-green and yellow-blue information and then applies spatial operators to those planes that reflect properties (measured by the contrast sensitivity functions) of the visual system before computing CIELAB colour differences at each pixel location. This spatiochromatic processing attempts to take into account the fact that the yellow-blue image signal in the visual system is undoubtedly at lower spatial resolution than the red-green or luminance information. For patterned regions of the image, the reproduction errors measured using S-CIELAB correspond to perceived colour errors better than errors computed without the spatial extension. An important advantage of this model is that if it is presented with two uniform coloured fields then the model behaves exactly like a standard colour-difference equation.

The key feature of Daly's visible-difference predictor is a model of the human visual system that concentrates on the lower-order processing of the system such as optics, retina, lateral geniculate nucleus and some higher-order processing in striate cortex. The model contains three main components: (i) an amplitude nonlinearity (in image-processing terms, a point process), (ii) the contrast sensitivity functions (implemented as a filter process), and (iii) a detection process which models masking processes within the visual system.

#### 4.7.2 Models of colour constancy

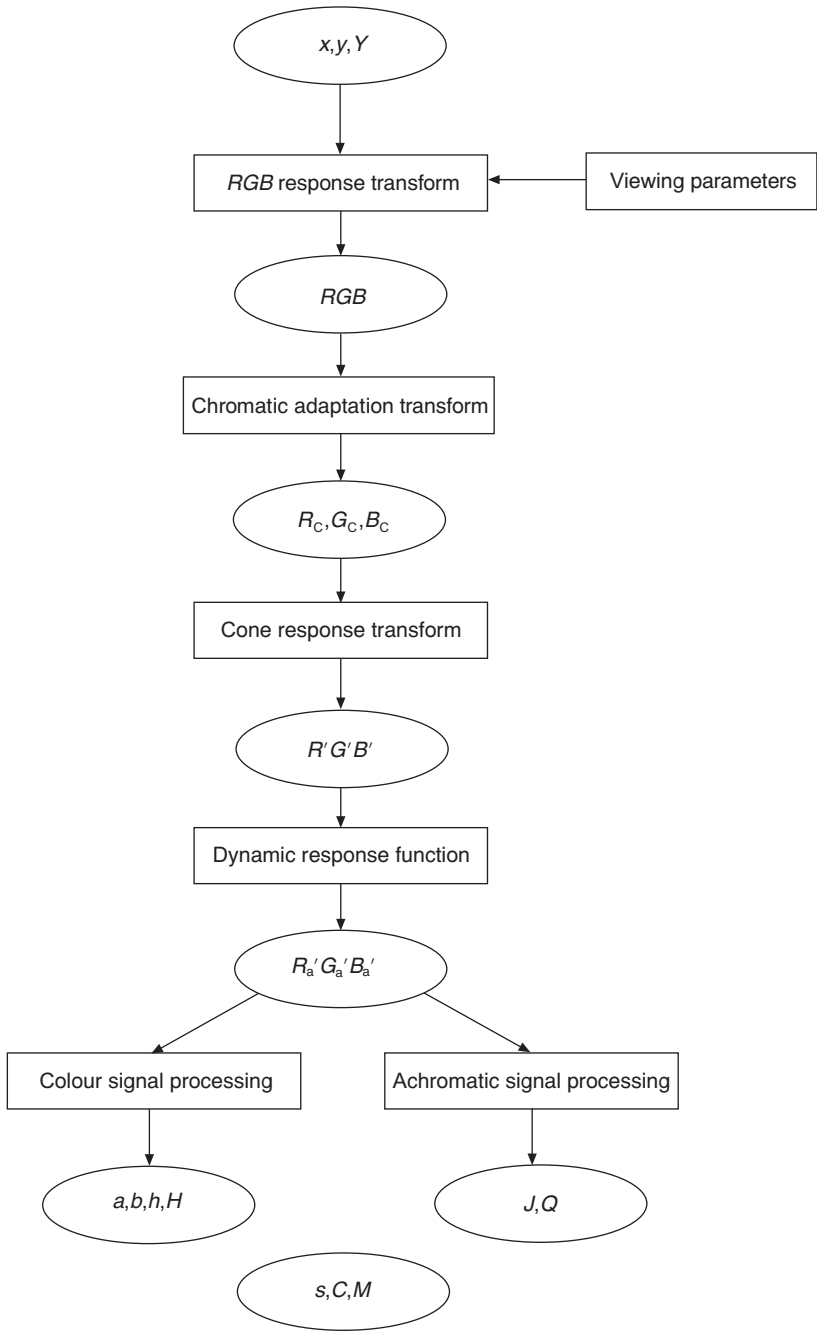
The most popular implementation of the von Kries rule holds that the coefficients  $k$  (Equation 4.2) are adjusted to keep the adapted appearance of a reference white surface constant (Worthey and Brill, 1986). Thus, for example, if the cone absorptions of a perfect white under a given illuminant are  $\Phi_{i,w}$ , then the adapted response for a surface under a second illuminant will be given by Equation 4.2 where the coefficients  $k_i = 1/\Phi_{1,w}$ . Note that under this scheme the adapted response for a perfect white will be 1 for each cone class under any illuminant. This particular implementation requires the visual system to know somehow which surface, if any, is white and may well fail if there is no white surface in the scene or if the white surface is incorrectly identified. It is interesting that the first stage of the transform from XYZ values to CIELAB colour coordinates involves normalising the XYZ values for a sample by the XYZ values of the illuminant so that  $L^*$  is 100 for a perfect white under every

illuminant. This normalisation process, which is loosely based upon a von-Kries-type scaling principle, allows the CIELAB colour coordinates to predict the colour appearance of surfaces under different light sources better than the tristimulus coordinates. CIELAB can therefore be considered to be a colour-appearance model. The transform allows CIELAB coordinates to predict perfect colour constancy for perfect reflecting diffusers since  $L^* = 100$  and  $a^* = b^* = 0$  for such samples irrespective of the illuminant. Of course, CIELAB is a relatively poor colour-appearance model; it overestimates the colour constancy of perfect white surfaces (it is known that colour constancy breaks down under substantial changes in the light source) and underestimates the colour constancy of chromatic surfaces under small changes in the illumination. Furthermore, the CIELAB system makes no attempt to predict spatial (e.g., simultaneous contrast) or temporal (e.g., successive colour contrast or after images) colour-appearance phenomena.

### 4.7.3 CIECAM97s

In 1996 the CIE were asked to recommend a colour appearance model for general industrial application (Luo, 1999). The agreed approach was to examine existing colour appearance models, to combine the best features of these models into a high-performance model for general use, and to test its performance against available experimental data. The result of this work was a model that was adopted by the CIE in Kyoto in 1997 and named CIECAM97s (Luo and Hunt, 1998a). The CIECAM97s model was tested using 700 pairs of corresponding colours from 14 data sets and 5,000 samples from seven magnitude estimation data sets. It was shown to perform better than, or equal to, any existing model at that time (Luo and Hunt, 1998b). CIECAM97s comprises four parts: a cone response transform, a chromatic adaptation transform, a dynamic response function and colour spaces formed by different combinations of colour appearance attributes (Fig. 4.4).

The first step of CIECAM97s is to convert the colorimetric values ( $x$ ,  $y$ ,  $Y$ ) to  $RGB$  signals via a linear transform. This is followed by transforming them to  $R_C$ ,  $G_C$  and  $B_C$  signals via a chromatic adaptation transform, which is capable of predicting the corresponding colour from the test to reference illuminant (an equal energy illuminant with  $x = y = 0.3333$ ). Recall that a pair of corresponding colours appear the same when viewed under test and reference illuminants. The CMC 1997 chromatic adaptation transform (CMCCAT97) is used in CIECAM97s. The  $R_C$ ,  $G_C$  and  $B_C$  signals are then transformed to cone responses. The dynamic response function is then used to predict the extent of changes of responses due to different luminance levels. The predicted adapted cone responses are used to calculate colour difference signals:  $a$  (redness-greenness) and  $b$  (yellowness-blueness), and  $A$  and  $A_W$ , achromatic signals for sample and reference white respectively. Subsequently, the  $a$  and  $b$  values are used to calculate hue angle and hue composition. The achromatic signals are used to compute lightness and brightness. Finally, the saturation, chroma and



**Fig. 4.4** The structure of the CIECAM97s transform.

colourfulness values are obtained. According to different applications, different colour spaces can be constructed by using different combinations of perceived attributes.

CIECAM97s can predict the effect of the colour of a background on the colour appearance of a stimulus and can also take into account the brightness of the viewing conditions. However, it cannot predict more complicated spatial effects such as White's effect or complex illuminant effects such as Helson-Judd.

#### **4.7.4 Cross-media colour reproduction**

There is great interest in being able accurately to reproduce colour on a range of different media such as computer monitor, print, and projection systems. Colour-appearance models are proving useful in achieving this aim. A general consensus has been formed that a five-stage transform is required to achieve successful cross-media colour reproduction. The transform includes four major components: a colour appearance model including a forward and a reverse mode, a device characterisation model for converting between the colour primaries of particular imaging devices and the CIE specification, a device profile for defining a translation from a device characterisation under a set of viewing conditions to a standard colour space under a reference set of viewing conditions, and a gamut mapping model for mapping out-of-gamut colours from the input to output devices while preserving overall appearance. The colour-appearance model plays a very important role in the forward and reverse stages of the five-stage transform. Colour-appearance models also provide uniform colour spaces in which to perform colour gamut mapping.

### **4.8 Future trends**

Methods for assessing the colour appearance of surfaces are becoming increasingly important for the food industry. The original CIE system of colorimetry was designed in 1931 for colour specification and is not suitable for the prediction of colour appearance. Transforms of the CIE tristimulus values, such as CIELAB, can be used to predict colour appearance but performance is often quite poor. Colour appearance is a complicated phenomenon that includes both spatial and temporal components but some advanced colour-appearance models are now available that can model some of the more straightforward aspects. For example, the CIECAM97s system can take into account the effect of a simple surround colour on the appearance of a coloured patch (simultaneous contrast) and the effect of the brightness of the viewing condition (brightness adaptation). However, much work is required before a colour appearance model will be able accurately to predict a wide range of colour appearance phenomena. It is important to recognise that although special figures are often used to illustrate specific colour-appearance phenomena the effects are ubiquitous in everyday vision.

## 4.9 References

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## 5

# Colour measurement of foods by colour reflectance

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### 5.1 Introduction: food colour and quality

The first judgement of a food's quality is more often than not dependent on its various appearance characteristics, such as colour, surface structure and shape. Colour is particularly important yet not so easy to define. Francis and Clydesdale (1975) described it as the sensation experienced when energy, in the form of radiation within the visible spectrum, falls upon the retina of the eye, but this definition does not give the full picture. Within the retina, rod-cells and three different kinds of cone-cells send signals to the brain which are then further processed in order to rationalise the colour of an object. Rationalisation happens regardless of changes in the level and colour of the surrounding lighting and allows objects to be recognised as having virtually the same colour under very different conditions. Over the past few years, many workers have tried to understand and develop models in this area of colour constancy but as yet it remains an approximate quality. This is one of the reasons for resorting to instrumental methods for measuring colour under standardised conditions.

Overall, food quality may be considered in terms of taste, texture, mouthfeel and other sensorial attributes or in terms of macro- and micronutrient content. For any concept of visual quality, colour acts as a very useful and intuitive indicator. Colour can be assessed at various times during the lifetime of a food product – from raw materials, e.g., the quality of flour for baking, through processing, e.g., monitoring roasting of coffee beans, and at the end point where a finished product needs to be confirmed as suitable for its designed purpose, e.g., sweets for decoration or powders for rehydration. In many cases, colour measurement provides a means of characterisation and quantification using

methods more simple and user-friendly than time- and labour-intensive compositional analysis or sensory evaluation.

There are a bewildering variety of methods and instruments available to the food technologist in the field of colour measurement. When one is approaching the subject for the first time or when attempting to devise a method for a material outside the normal experience, the wealth of possibilities available sometimes makes the choice difficult. It is the purpose of this chapter to attempt to identify a systematic approach in order to ease the task. The approach is concerned primarily with the use of tristimulus colorimetry and reflectance spectrophotometry. These two techniques will be discussed in detail along with colour scales and formulae commonly in use. The key points which may influence measured results will also be described followed by advice on the selection, preparation and presentation of samples. Finally, detailed references to work carried out in the field of food colour measurement and where to access further information will be provided.

## 5.2 Colour measurement principles and methods

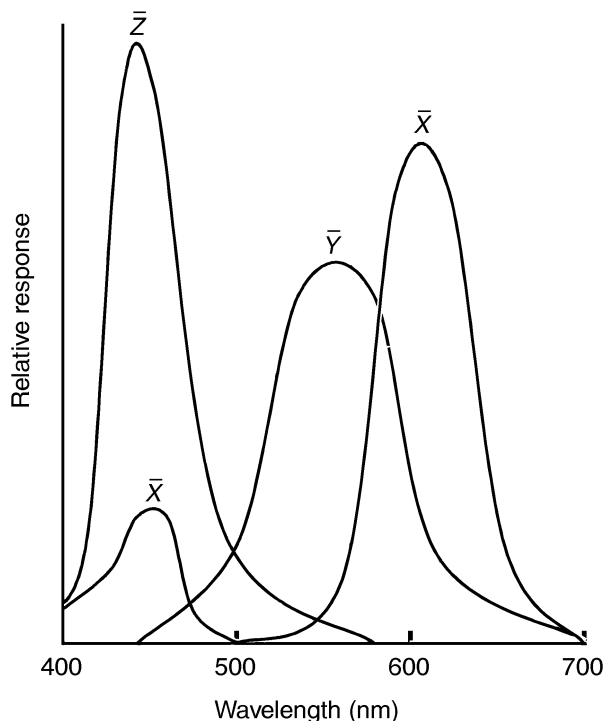
In order to understand the principles of colour measurement by reflectance we must appreciate how our foods attain their visual properties. Incident light is reflected from an object in a way determined by its physical and chemical properties. The presence of chromophores will cause the specific absorption or scattering of certain wavelengths of light leaving the remainder of the incident light to travel onwards to the 'observer'. In some cases, the physical nature of the sample may prevent light interacting with the chromophores or in other cases cause it to be trapped inside the sample. Whatever the mechanisms responsible, only light reaching the 'observer' may be measured and quantified.

### 5.2.1 Tristimulus colorimetry

The scientific basis for the measurement of colour is the existence of three different types of response signals in the human eye. Though four different types of receptor –  $\rho$ ,  $\gamma$  and  $\beta$  cones, and rods – have been identified, the messages from these are encoded (in a way still not fully understood) to give three types of signal.

In historic work carried out in the late 1920s and early 1930s by Wright (1969) and Guild (1931), the performance response characteristics of a standard human eye (the standard observer) to different spectrum colour light sources were established. This work formed the basis of the Commission Internationale d'Éclairage system (CIE 1931). In order to be able to relate the results obtained from different combinations of primary colour stimuli, linear transformation equations were used to convert Wright and Guild's curves to more usable functions. This resulted in the definition of three integral primaries,  $X$ ,  $Y$  and  $Z$  (see Fig. 5.1). These primaries do not exist as real lights, but they encompass all colours and are, therefore, mathematically very useful.



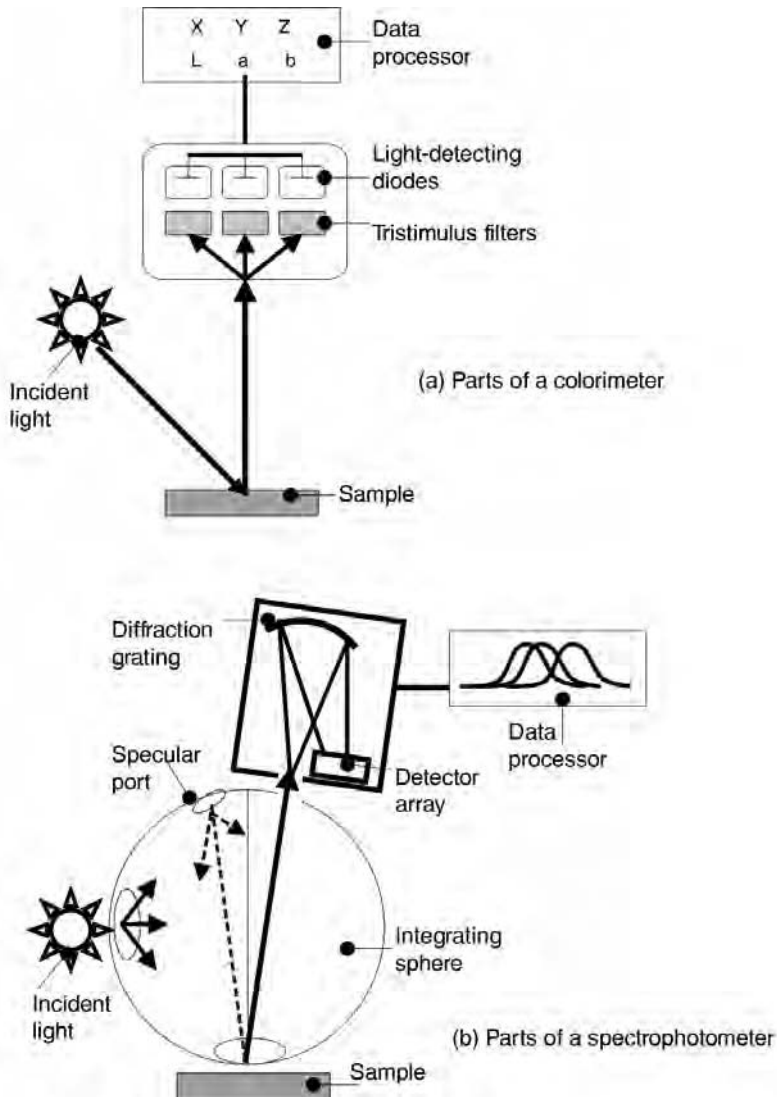


**Fig. 5.1** Colour matching response functions of the human eye for the original CIE 2° observer.

In a tristimulus colorimeter, three or four filters duplicate the response of the standard observer. The filters, which correspond to the three primary colours in the spectrum (red, green and blue), can be combined to match most colours. The more sophisticated instruments carry a fourth filter to simulate the blue part of the CIE  $[\bar{X}]$  function shown in Fig. 5.1. The other essential parts of a tristimulus colorimeter are a white light source, an array of photometers and, nowadays, a computer or an interface to one, as shown in Fig. 5.2a. The computer can collect responses as well as carry out data transformations between CIE and other colour scale systems or between different standard white light sources or white diffusers. Hunter and Harold (1987) give a good summary of the transformation formulae employed. Data from a colorimeter is given as a three-point output, commonly CIELAB, HunterLab or  $Y, x, y$ .

### 5.2.2 Reflectance spectrophotometry

Unlike the colorimeter's filter system, a spectrophotometer works by measuring the whole spectrum of visible light reflected from a sample, i.e., between 380nm and 700nm. Commonly, measurements are taken, via an integrating sphere and then a diffraction grating (see Fig. 5.2b) with the results being expressed as the



**Fig. 5.2** Instruments for colour measurement by reflectance.

ratio between the reflected light from the sample and that from a known reference standard. Different instruments measure at different wavelength intervals, depending upon the nature of the spectral analyser and control electronics; 10nm or 20nm are accepted standards. Since reflectance is calculated by means of the above ratio, it is commonly expressed as a percentage. Thus, a perfectly reflecting diffuser will have a reflectance of 100%. During measurement analysis, however, reflectance is usually expressed as a fraction, with the ideal white tile then having a value of 1. Conversely, a black

sample, which absorbs all incident light, will have a reflectance of 0% or 0. In between these two extremes fall the other visible colours. If a sample absorbs all but red light, it will show high reflectance values in the 'red' region of the reflectance spectrum.

A spectrophotometer 'captures' all surface-reflected light across the visible wavelength range and thus can graphically analyse and output what the perceived colour should be. Every surface colour can therefore be characterised by a reflectance curve. It should be noted that certain materials, often those with added brightening agents or a natural fluorescence, may record a reflectance of greater than 100%. An important characteristic of modern spectrophotometers is that they are dual-beamed. The use of the second, or reference beam, is important in ensuring stability and minimising error. The reference beam is light from the sphere walls which is collected at a reference port and directed via a mirror to another spectral analyser identical to the first. The resulting reflectance value output from the spectrophotometer, therefore, is a ratio rather than an absolute value and any drift resulting from electronic or physical error will be cancelled out.

Where measurement error is often encountered is during the definition of the measurement geometry, that is to say, the relationship between the incident (source) and reflected (measured) light. Most spectrophotometers, which are sphere-based instruments, allow the reflected light to be measured either with or without its specular component. As it is the surface properties of the sample that determine the amount of specular reflection, different results will be obtained when measuring a very rough surface as opposed to a highly glossy one. Which specular mode to choose will depend upon the intended application. Colour-only data, say for end-product quality control, may require a specular-included measurement; whereas if the intention is to correlate instrumental with sensory results a specular-excluded result may be more representative of what the eye sees.

One of the disadvantages of using a sphere-based spectrophotometer, as opposed to a 0°/45° spectrophotometer, is that the measurement port is open and unprotected allowing direct access to the sphere itself. There is therefore the potential for samples to fall into the instrument and contaminate the white diffuse sphere wall. Some workers have tried to protect the sphere by, for example, placing a thin film between sample and port (Pauletti *et al.* 1998) or using an opti-glass window. In either case, the optics of the system will be disrupted and any results obtained must be considered as relative. Despite the greater cost of a spectrophotometer in comparison to the simpler colorimeter, the ability to measure full reflectance curves allows colour-matchings to be made, tolerance volumes to be defined and a more in-depth understanding of many colour change processes to be achieved. Several manufacturers produce their instruments in both bench-top and portable models facilitating both lab analysis and near-line studies.

### 5.2.3 On-line colour measurement

The two commonly used instruments discussed allow both laboratory and near-line colour measurements to be made. With the advent of almost fully automated lines, true on-line colour measurement would be of great value in many factories. McFarlane (1988) discusses the economic benefits of on-line and closed-loop systems. His paper highlights closer adherence to specifications, minimisation of waste during start-up and product change, as well as an increase in process efficiency and plant throughput as key issues.

Work at the author's laboratory (P. Joshi) has recently focused upon the use of fibre-optic reflectance probes to generate on-line data. Several fibre-optic cables may be bundled so as to provide both an illumination source and collection point in one probe. This probe is connected to a defined light source, such as a Xenon or Halogen lamp providing D65 illumination, and then output to a spectrophotometer to generate full reflectance curves. The inclusion of a 45° optical window facilitates the 0°/45° viewing geometry. The whole probe is then inserted into a pipe to measure the product during its actual processing. No sampling procedure is required and with the appropriate software, instantaneous CIELAB or XYZ data may be acquired. As with all colour measurement systems, calibration is of key importance, as is the need to ensure that the pipe-fitting provides an adequate optical path-length without the interference of extraneous light. Such in-line systems have been previously used in the petroleum industries but with further investigation and development their routine integration into food processing could be a valuable future tool for both quality control and process validation.

Other means of spectroscopically obtaining on-line data have been investigated by Apruzzese, Balke and Diosady (2000) whose fibre-optic system measures in the Vis-NIR range to monitor both colour and composition of extruded cornflour. Furthermore, the continuing advance of digital camera technology has enabled several workers to carry out reflectance colour measurement using so-called 'Computer Vision Systems' (Wang and Sun 2001). Such techniques may use commercially available software such as Adobe PhotoShop to carry out the image analysis (Papadakis 2000) or use custom-made interfaces. In both cases the basis is to convert the captured RGB values (the colour model used in digital camera and computer monitor displays) into the more widely understood CIELAB system.

### 5.2.4 Colour scales and colour difference formulae

As mentioned previously, the now routine integration of a pc means that the data collected from either a colorimeter or spectrophotometer can be transformed to XYZ, CIELAB, HunterLab or other colour system as specified by the user and provided by the software. The initial step from instrument response to XYZ requires the definition of standard observer functions.

The first standard observer was defined by the CIE in 1931. This was based upon a series of colour-matching experiments carried out four years earlier and

involved using a 2° field of view. This was thought to be adequate since in the human eye, the receptor cones, which are responsible for colour vision, were concentrated at 2° around the fovea. It was later established that this was, in fact, quite unrealistic as most visual assessments are done with a field of view much greater than 2°. Thus, in 1964 the CIE introduced the 10° Standard Observer – the 10° encompasses the area in the fovea that contains the rod receptors (responsible for lightness discrimination) and so is closer to human vision. Many earlier studies have been carried out using the 2° observer and so caution must be exercised if comparing these values to those measured using the now more common 10° standard observer. Most software packages have the capabilities to convert data between the two or for those programming their own systems the raw data (chromaticity co-ordinates and colour matching functions) are available in the international standard ISO/CIE 10527 ‘CIE standard colorimetric observers’.

The aim of the more popular of the colour scales is to represent colours in a three-dimensional colour space, such that similar visual colour differences are represented by approximately similar distances in the colour space. Such colour scales are known as uniform scales. The original CIE scales, represented diagrammatically in Fig. 5.3, were not intended for identifying the colours of objects and are certainly not uniform in spacing colours according to their visual differences. Over the last forty years the *Commission Internationale de l'Éclairage* (CIE) in particular have worked to try to stem the confusion caused by the presence of the many formulae in use and standardise colour space. They managed to combine the opponent chromatic attributes used by Hunter and Schofield, with the MacAdam cube-root simplification of the earlier Judd polynomial. The result was the publication of CIELAB (or CIEL\*a\*b\*) in 1976 (Smith 1997). At this point, the asterisk superscript was introduced in order to differentiate the CIELAB parameters from other similar ones still in use.

$$\begin{aligned}
 L^* &= 116f(Y/Y_n) - 16 \\
 a^* &= 500[f(X/X_n) - f(Y/Y_n)] \\
 b^* &= 200[f(Y/Y_n) - f(Z/Z_n)] \\
 C^* &= \sqrt{a^{*2} + b^{*2}}
 \end{aligned}
 \tag{5.1}$$

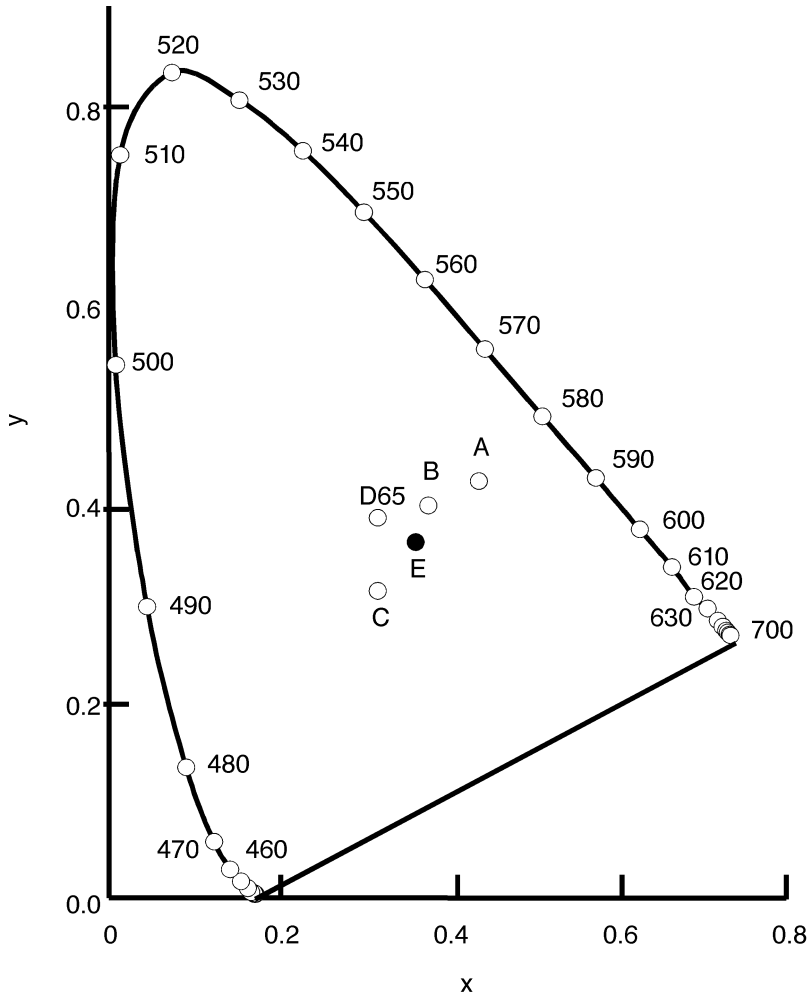
where

$$f(I) = \begin{cases} I^{1/3} & \text{for } I > 0.008856 \\ 7.7871 + 16/116 & \text{otherwise} \end{cases}$$

thus

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Work to create a truly uniform colour space still continues. Tolerances based on  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta b^*$  terms give too much area out of visual tolerance but within



**Fig. 5.3** CIE  $x, y$  chromaticity diagram: locations of illuminants A, B, C, D65 and E (equal energy) are also shown.

instrumental tolerance. Using lightness, chroma and hue ( $L^*C^*h^\circ$ ) attributes allows ellipsoidal tolerance areas to be derived which are much better and easier to sensorially describe than the vague redness-greenness, yellowness-blueness opponent pairs.

In 1994 following extensive studies by the Colour Measurement Committee (CMC) the CIE94 *total colour-difference* was defined. In the equation, lightness, chroma and hue are weighted and corrected to account for variation in perceived colour magnitude and for sensitivity and variation in experimental conditions. Recently, the most prominent UK workers in the area of colour equation development combined under a technical committee to determine a generalised and reliable formula (Luo *et al.* 2000). They have also come up with an equation

based on CIELAB which includes not only weighted functions but also an interactive term for chroma and hue differences. This new equation, CIEDE2000 has now been officially adopted by the CIE. All the advanced colour difference formulae (including CIE94 and CIEDE2000) based on CIELAB may be generically described by the equation 5.2.

$$\Delta E^* = \sqrt{\left(\frac{\Delta L^*}{k_L S_L}\right)^2 + \left(\frac{\Delta C^*}{k_c S_c}\right)^2 + \left(\frac{\Delta H^*}{k_H S_H}\right)^2} + \Delta R \quad 5.2$$

where

$$\Delta R = R_{Tf}(\Delta C^* \Delta H^*)$$

and  $\Delta L^*$ ,  $\Delta C^*$  and  $\Delta H^*$ , are the CIELAB metric lightness, chroma and hue differences respectively

$\Delta R$  is the interactive term between chroma and hue differences

$S_L$ ,  $S_c$  and  $S_H$  are the weighting functions which vary depending upon the position of the sample pair being considered in CIELAB space

$k_L$ ,  $k_c$  and  $k_H$  are the parametric functions which are adjusted according to different viewing parameters.

Much of our analysis in the food industry rarely requires accurate colour matching or prediction and, hence, these more advanced colour equations may be too specific for our needs. Use of the CIEL\*a\*b\* and CIE94 is growing in popularity however, especially when correlation with sensory analysis is required. Relating sensory colour analysis with instrumentally measured data is covered in more depth later in this book. Of practical note, it is recommended that users comparing data confirm the colour notation and system used – the lightness, and chromaticity co-ordinate used in the Hunter-Judd analysis  $L$ ,  $a$ ,  $b$  do not correspond exactly with the L\*a\*b\* of CIELAB. Consequently, it is essential that full specification including colour system, illuminant type and viewing geometry is quoted when publishing colour measurement data. If conversion is required many web-based applications exist within which one can convert data from one colour system to another, e.g., CIELAB to HunterLab to Munsell Chip Notation ([www.colorpro.com/info/tools/convert.htm#TOP](http://www.colorpro.com/info/tools/convert.htm#TOP)).

### 5.3 Colour measurement methodology

Methods for the instrumental measurement of food colour by reflectance involve four stages:

- 1 standardisation of the instrument
- 2 setting the instrument variables
- 3 selection, preparation and presentation of the sample to the instrument
- 4 determination of the colour values.

There are many variable factors in each of these four stages which can affect the final result. In order to be able to compare results for a particular food material, these variables should be recognised and efforts made to eliminate, or at least reduce, their effects.

Colour standards provide the references against which the colours of materials can be instrumentally compared. They fall into two classes, primary standards and secondary standards. Primary standards are pressed powder tablets of fresh  $\text{MgO}$ ,  $\text{BaSO}_4$  or halon G-80 (pressed tetrafluoroethylene resin manufactured by the Allied Chemical Corporation) maintained by governmental standards agencies such as the National Bureau of Standards in Washington DC, USA or the National Physical Laboratory in Middlesex, England. These white standards are measured against a theoretical perfect white diffuser by means of an auxiliary sphere to derive an absolute reflectance value. Unfortunately, at the moment there is not a complete consensus between the various standards institutes on the perfect white diffuser values.

Secondary standards can be subdivided into a number of groups. Hunter and Harold (1987) have described seven types of secondary standards in the following terms:

1. Master standards calibrated by reference to primary standards and usually in the form of porcelain or opaque glass panels, ceramic tiles, acrylic painted chips, etc. These are maintained with extreme care, usually at the standards institutes, and are used only to calibrate other secondary standards.
2. Working standards used as a reference for a group of laboratory instrument standards. These are usually ceramic or porcelain tiles.
3. Instrument standards used to calibrate a particular instrument.
4. 'Hitching-post' standards which are used in place of a white standard and which are close in colour to that of the samples to be measured. This technique is used to minimise errors caused by scale-interval defects in the instrument.
5. Specific calibration standards which are permanent specimens or standards used for measurements carried out on impermanent products. To derive these standards the impermanent product is first measured on a reference instrument. The product can then be used to calibrate a second instrument on which the permanent standard is also read. This reading then becomes the assigned value of the permanent standard.
6. Diagnostic standards used for instrument checking or fault finding in inaccurate instruments.
7. Material comparison standards, which are usually samples of the materials under examination and provide limiting or target values.

It can be seen that, in all except the last two cases, the colour values of the standards are traceable, via the working and master standards, back to the primary standard.

Before a standard is used to calibrate an instrument, the instrument should be thoroughly warmed up and stabilised. Colour standards should be placed under



the instrument port in the same manner each time a calibration is carried out, to avoid the effects of any surface directionality in the colour layer of the standard on the calibration values. Because some of the colour standards exhibit thermochromicity, i.e., their colour values change as the temperature changes, it is advised that calibration be carried out with rapidity in order to avoid the instrument lamp raising the temperature of the standard. The subject of thermochromicity of the colour standard has been described by Brimelow (1987) with reference to measurement of tomato colour.

Kent and Smith (1987) reported the results of an inter-laboratory study on measurement of colour standards. They came to the obvious and important conclusion that in order to transfer and compare colour data from one laboratory to another, which is certainly necessary when colour is being used as a buying criterion, the measuring system and the colour standard have to be carefully defined. Most instrument manufacturers will provide details of best practice with respect to how the instrument and its standards should be maintained.

### **5.3.1 Setting the instrumental variables**

The instrumental variables include the choice of light source, the measuring geometry and the relative sizes of the specimen area and the illuminated area. When any colour measurements are made, it is necessary to specify the illuminant used as part of the viewing conditions. In 1931 the CIE established various standard illuminants that have characteristics close to natural light sources but that can also be reproduced easily for use in the laboratory or in instrumentation. Illuminant A represents light close to that given by a tungsten filament lamp, which might for example be operating during subjective assessments of colour in the home. Illuminant B represents direct sunlight, whereas illuminant C represents average daylight from the total sky, which again might be present during assessments in the home. More recently the CIE (1979) proposed a D series of illuminants which more closely represent daylight than do B or C and also incorporate an ultraviolet range. The D series correlates with various daylight colour temperatures; the most commonly used is the D65 illuminant, approximating to a colour temperature of 6500°K.

It should always be borne in mind, however, that if a subjective assessment of the colour of a particular food is normally carried out under, say, a tungsten filament light or under direct sunlight, then it may be preferable to carry out the instrumental measurement under similar lighting conditions. It should be noted also that a pair of samples which colour-match another under one illuminant may not match under a different illuminant. This phenomenon is known as metamerism and is of great importance to those in the textile and paint industries.

The choice of the remaining instrumental factors, such as measuring geometry and the relative sizes of the specimen viewing area and the illuminated areas, are very much influenced by the optical classification of the specimen. In order to determine the colour values of a product, therefore, it is necessary to

decide whether the prepared sample is opaque, translucent or transparent. Transparency is relatively simple to assess; when a clear image of a light source can be seen through the sample it can essentially be considered as transparent. Translucency is more difficult to determine as the dividing line between opacity and some translucency is imprecise. A simple test is to measure the specimen with maximum area of illumination and maximum viewing aperture and then to repeat the measurement using the same viewing aperture but a reduced area of illumination. If there is a large increase in the lightness reading (the  $L^*$  value in the CIELAB scale), then the product is translucent. Another good indicator is to measure the sample over firstly a black then a white background. If these two measurements are the same, then the sample can be considered to have an infinite optical thickness.

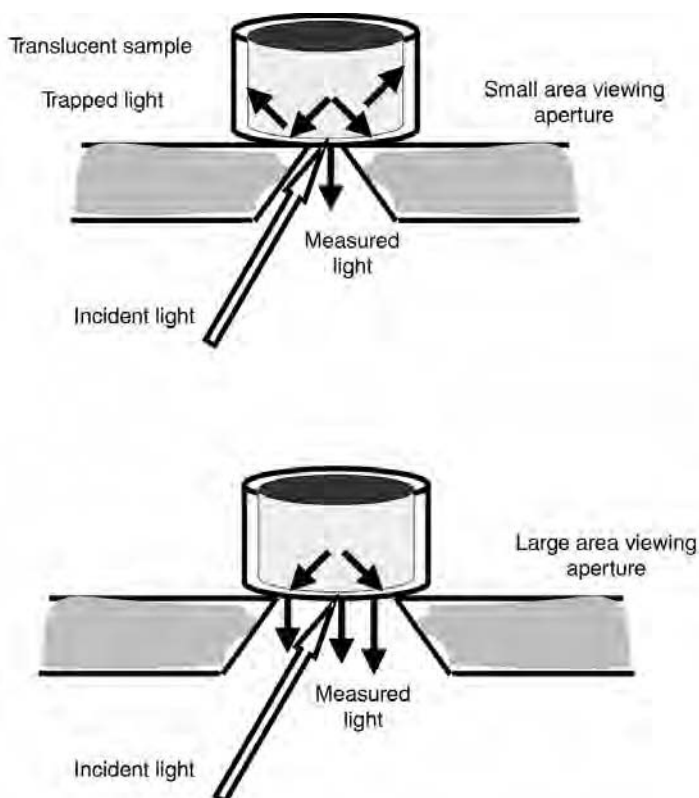
The viewing geometry must also be specified as part of the instrumental conditions. It is generally agreed that with food samples most applications can be covered without much compromise using  $0^\circ/45^\circ$ ,  $45^\circ/0^\circ$  (source/detector), or specular component excluded (SCE) viewing geometry. This is the preferred geometry when examining the reflected colour of translucent samples. It is also satisfactory for opaque samples, provided the samples being compared are of equal texture or gloss. Even samples with different texture or gloss characteristics can be compared using this geometry, provided that appearance differences rather than simply colour differences are being assessed. SCE geometry should be used for transparent samples or for measuring transmittance through translucent samples.

One source of error arising from the viewing geometry occurs through failing to define whether specular reflectance is included or not. With  $0^\circ/45^\circ$  or  $45^\circ/0^\circ$  geometries, very little specular reflectance is included in the total reflected light detected, even with glossy but non-smooth samples. However, with spherical ( $d/8^\circ$ ) geometry, specular reflectance will be present in the reflected light from glossy samples and the decision has to be made to include it or exclude it.

The choice of illuminated area size and aperture size is also very dependent on the nature and the optical class of the specimen. Small area illumination is used on small samples, on samples having only small areas of flatness and on highly translucent and uniform samples. In the latter case, Hunter and Christie (1978) have recommended that the small area illumination be used together with large aperture viewing in order to capture that proportion of the light scattered by the sample and then returned to the detector from outside the illuminated area (see Fig. 5.4). Particulate solids are best measured using large area illumination and large viewing apertures.

### 5.3.2 Selection, preparation and presentation of the samples

The sample itself is the key to good colour measurement. In optical terms, samples can be divided into four main classifications:



**Fig. 5.4** Translucent foods diffuse the incident light resulting in lost measurement. Using a larger area viewing aperture allows this trapped light to be measured.

1. opaque objects
2. metal-like objects
3. transparent objects
4. translucent objects.

With opaque objects, a class into which most food materials fall, light is either directly reflected from the surface as a white highlight (gloss) or absorbed, scattered and diffusely reflected from the surface (perceived colour). Opaque materials do not transmit light. The relative amounts of gloss and colour perceived depend, of course, on the surface properties, but also on the viewing angle. In order to compare colour differences independently of the gloss characteristics, the optimal positions of the light source and the detector (e.g., the eye) relative to the sample surface are  $45^\circ$  and  $0^\circ$  or  $0^\circ$  and  $45^\circ$ . This optical arrangement is replicated in many tristimulus colorimeters and therefore these instruments measure sample colour and not surface effects. Most spectrophotometers, however, provide data either with or without this specular effect and if managed correctly can provide useful information on product shine or packaging influences.

In the case of metal-like objects, virtually all the light is reflected as gloss and very little is diffused. Very often the reflectance is at a dominant wavelength, which provides colour gloss, rather than at all wavelengths which would provide white highlights. Foods, though they may sometimes have a high gloss component, rarely fall into this class of metal-like materials.

Transparent objects exhibit colour by regular transmission, that is, they absorb light and then transmit it preferentially at different wavelengths along the path of the light beam. Some liquid food materials and a few solid food materials are transparent, such as vegetable oils, fruit serums and wines, certain sugar confectionery, fruit jellies, etc.

The class of materials between transparent and opaque, to which many food materials belong, is called translucent. Some of the light falling on a translucent object can pass through it in the direction of the light, but the remainder is diffused within it and emerges in different directions. As a consequence, the size of the measurement aperture can greatly influence the amount of collected light (see Fig. 5.4). The light-scattering properties of hazy or turbid food materials are a complex subject which has been covered in some depth by MacDougall (1982; 1983; 1987). It is clear that with this class of material, instrument/object geometry plays a large part in the colour values obtained. MacDougall (1987) has illustrated this point using concentrated and diluted orange juices as test samples.

When reflectance measurements are carried out on opaque or translucent food materials, a number of problems exist in the selection and preparation of the samples. The first is that simply by reason of its presentation method, the sample area as presented to the instrument may not be representative of the bulk of the material under test. Most instruments in common use are designed to accommodate samples which are flat, and therefore reflect light from one plane only. Most food materials are not flat and some degree of compromise has to be made in order to create viewing areas which are. It is often the case that it is advantageous to deliberately change the surface characteristics of the sample in order to enhance colour differences between specimens. André and Pauli (1978), for example, pulverised dehydrated pasta into a powder and then tabletted the powder for colour measurement in order to minimise variation caused by differences in the shapes and surface properties of commercially available products.

A second important problem is that other factors come into play which may affect surface reflection during the process of creating a flat viewing area. These factors include the ambient conditions of temperature and humidity applied during the process; the variables within the actual method used (e.g., the blade sharpness during cutting, the technique of polishing, the pressure applied during pressing); chemical reactions (e.g., browning effects) or physical reactions (e.g., settling effects) occurring at the surface up to and during the measurement event; and the settlement of vapour, dust or film-forming agents on the surface after its formation.

A third problem is the general non-uniformity of the surface colours of foodstuffs. This means that it may be necessary to make repeated measurements

in different places on a surface or make measurements of different samples from a bulk in order to achieve statistically significant average values. One ingenious solution to the problem of making repeated measurements on a surface was suggested by Francis (1952) for apples. By rotating a single fruit rapidly in a small viewing aperture, an average surface colour was obtained.

One further problem is the physical size of the food sample. Some specimens may simply be too small to measure successfully as single items, though recently instruments with very small aperture viewing heads which permit measurements on areas of around 3 mm diameter are available. It is, however, more usual to bulk such samples and then employ large area sample cups and large diameter instrument apertures when making the measurements.

When preparing samples it is important that the preparation procedure is strictly adhered to in order to ensure a high repeatability of measurement. Grinding, milling and mixing may all help to create sample uniformity but at the same time it must not be forgotten that such techniques will undoubtedly influence the sample's light-scattering properties (Giese, 2000). In some cases, the physical aspects of the sample can be more influential than the colorant concentration itself. Particle size, size distribution and component packing may influence the sample optics and hence, the measured colour (Joshi, 2000).

Apart from considering all of the above, it is also important to recognise that sample preparation and presentation may be influenced by the desired result of the colour measurement. For example, if the goal is to achieve a good correlation with sensory data, then it is essential that the instrumentally measured sample should be in the same state as that seen by the visual panel. If colour measurement is to be used as a buying criterion the trial sample must be presented as was the standard used to set the tolerance limits. Also, if a relationship between colour and some other physical parameter is to be made, e.g., particle-size or water-activity, both samples should undergo the same preparation for each test. Some general rules for the preparation of different types of food materials for reflectance colour measurement are discussed as follows.

#### *Opaque powders, granules and flakes*

It is usual to make multiple measurements on such materials and average the results. Bulk amounts are normally sieved prior to subsampling in order to reduce errors due to size variation in the particles. Hunter and Harold (1987) have clearly illustrated that the larger the particle size, the less the light is scattered and therefore the more coloured the sample appears. Smaller particles scatter and reflect more incident light and the sample therefore appears lighter. Many of the errors encountered in comparing the colours of powders and granules relate to particle size effects.

One of three methods of sample preparation of powders can be utilised. Samples can be poured into cups with transparent bases and measurements made through the base. Alternatively, samples can be poured into a container with sides of equal height and the top can be scraped flat with a straight edge.

Measurements are then made from above. Thirdly, a powder compactor can be used. This is a device which can create a disk specimen from a sample of known weight and bulk density by applying a standardised force. In this case measurements can be made from above or below. In any case where a sample is being measured through a 'window' it is essential that the material used is optically flat and transparent. It must be remembered that there will be some influence on the measured result since all materials have at least 4% specular reflectance – practically this may manifest itself as high lightness values and an overall increase in  $R\%$  across the spectrum. It is, therefore, essential to use quartz or opti-glass sample holders which will neither absorb nor reflect excessively in the visible range. If measurement through some other material is unavoidable, say when monitoring shelf-life through plastic packaging, then using specular component excluded geometry will go some way to reducing the packaging optical effects.

#### *Opaque particulate or lumpy materials*

Materials from 5 mm to 20 mm in size can be very difficult to measure with good repeatability, particularly if they are non-regular in shape. As has been mentioned, several instruments have recently appeared on the market with small diameter viewing heads, such as the Minolta CR 121 or the MacBeth ColorEye XTH. Normally, however, it is the practice to place particulate or lumpy materials into a large container with an optically flat and clear base, trying to ensure that the packing is as solid as possible. Measurements are then usually made from below.

An alternative method of presentation has been evolved which is useful in situations when repeated measurements have to be made on solid materials which have a high oil or fat content. These materials may smear or coat the surfaces of a glass cell. The method utilises clear PVC film (e.g., Saran Wrap). The film is stretched across the viewing port of the colorimeter and the sample is placed on the film. Measurement is made from below. After each measurement, the specimen can be quickly wrapped up in the film and discarded, thus eliminating the need for frequent washing of valuable glass sample cups (Mabon 1989).

#### *Large area solid foods*

When colour measurements are made on solid opaque foods, such as whole fruits or thick slices of red meat, the specimen field should be flat and uniform. If a natural flat surface is not available, then it is sometimes acceptable to form such a surface by cutting or pressing. The latter can be achieved by sandwiching the sample between two plates of glass, one of which should be optically flat and transparent. Viewing is carried out through the transparent plate. If the food does not lend itself to cutting or pressing, then one other possibility is to rotate the food object in front of the viewing port of the instrument (Francis 1952). Solid foods often show directionality. When the directionality is obvious, the specimen should be measured in the same orientation for each test. When the

directionality is haphazard or undefined, one useful technique is to turn the sample through 90° between the two readings and average the results.

Many solid foods are translucent, for example, cheeses, butters, fatty meats and high water content fruits and vegetables. The effects of translucency on colour readings are large, particularly when such materials are being measured in thin slice form (for example, bacon, other sliced meats and sliced fruits). It is essential to standardise the preparation procedures for such samples. Normally a standard white backing plate would be employed and the sample thickness would also be standardised. Specimens are often viewed through an optically clean and flat plate placed on the surface of the solid. Some of the effects of the preparative variables on the colour values of sliced meats have been discussed by MacDougall (1982). A few food solids are transparent and they can be measured by transmittance procedures. Specimens of known thickness are held against the viewing port of sphere-type colorimeters/spectrophotometers for measurement.

### *Pastes and slurries*

Food pastes and slurries are normally translucent materials. It is usual with such materials to introduce them into cells with optically clear and flat bases, to such a depth that all the light is trapped and diffused within. Measurements are made from below. In some instances, however, particularly when the aim is to compare lightness values in dark samples or when a standard background is desirable, a special cell equipped with a ring insert of known height is employed. The sample slurry is poured into the cell to fill it to above the ring and a dish with a white diffusing surface is pushed down to nest on the ring. This fixes the light path length of the sample when it is viewed from below.

Often it is advisable to cover the specimen cell with a black-lined box in order to prevent extraneous room light from passing into the specimen. Care should be taken when introducing pastes and slurries into test cells that no air is entrapped in the sample. When pastes and slurries are prepared by mincing followed by diluting with water, the mincing conditions must be standardised as must the dilution factor. Samples should be well stirred and then measured quickly to reduce the effects of sedimentation and oxidation. Finally, the thermochromic properties of certain food materials should not be forgotten, and sample temperatures should be standardised.

The effects of some of these preparative factors – dilution, test temperature and time, presence or absence of a black cover – on the colour values of tomato pastes have been discussed by Brimelow (1987). As already noted, MacDougall (1983) has illustrated the effects of the dilution factor on colour values in the case of orange juices.

### *Liquids*

Liquids can be translucent (milk, syrups, fruit juices, egg yolk) or transparent (wines, vinegars). Translucent liquids are measured in the same way as translucent slurries and pastes, and once again choosing the correct optical path-length can determine the validity of the measured result.

Transparent liquids are measured by pouring into optically clear fixed path length cells with parallel sides and then taking transmission readings with sphere-based instruments. Deeply coloured liquids such as dark treacles are measured in short path length cells of the order of 2 mm, whilst lightly coloured liquids such as white wines are measured in long path length cells up to 50 mm. Alternatively, using a cell backed with a white tile may permit colour measurements by transreflectance. In such cases the effective path length becomes twice that of the cell since the illuminant light travels through the sample, reflects from the white tile or mirror backing, and then traverses again through the sample before being measured.

### 5.3.3 Interpretation of colour values

The last stage in obtaining a colour measurement is the conversion of the raw signal to colour values in one or other of the colour scales. In modern instrumentation, software packages are capable of carrying out these conversions with speed and accuracy, and can even make calculations to predict comparative colour results for different light sources.

Colour may be expressed as full reflectance curves or by the chosen colour space, most commonly CIELAB or HunterLab. Which colour output to choose depends, again, on the desired application. Full reflectance curves can be very useful if dynamically monitoring a process or if trying to create some calibration linking colour to another physical parameter. Reflectance data is also essential if using Kubelka-Munk theory in dealing with the optical properties of thin layers.  $L^*a^*b^*$  data is very good for monitoring colour trend during production and most modern systems will also allow tolerance setting to warn the operator if some production fault occurs.  $L^*C^*H^*$  may be more useful if the aim is to correlate instrumental measurement with sensory analysis. Converting this kind of data to Munsell notation is also very valuable especially if correlation to sensory data where Munsell chips have been used as references are required.

## 5.4 Colour measurement of typical food materials

In this section, some typical applications of food colour measurement will be described by reference to practical work in this area. The work will be treated under the headings utilised earlier in the chapter: powders, granules and flakes; particulate and lumpy materials; large area solid foods; pastes and slurries; liquids. For further details on the preparation procedures, the sample temperature, the sample presentation, the instrument illumination and aperture diameters, the colour standard and the colour scale employed in each case, the reader may refer to Tables 5.1 to 5.5, respectively. The instruments utilised in each case are briefly summarised in Table 5.6 on page 108.



## 5.5 Powders, granules and flakes (Table 5.1)

Johnston *et al.* (1980) reported that several researchers had compared colorimetric values with visual judgements for durum semolina, but that no one had compared colorimetric data with extracted pigment content. They found that semolina reflectance *b* values were highly correlated with pigment content as determined by pigment extraction and estimation using the standard AACC water-saturated butanol method, particularly when the objective was to segregate highly pigmented semolinas from low pigmented ones. The authors pointed out that reflectance colour measurement in these types of applications could be a genuine time-saving alternative to the lengthy pigment extraction technique.

One of the major problems with these kind of products is the effect of particle size. In order to minimise grind size effects in ground coffees of different roasts, Little and MacKinney (1956) standardised on a very fine grind with maximum lightness value. Seakins (1971) tackled the problem by another means and developed a method to measure the colour of casein by immersing it in a liquid of similar refractive index. Reflectance measurement of dry sample preparations resulted in poor separation of colour grades because reflectance increased as particle size decreased. However, when transmittance values were measured on the liquid suspension, the colour grades were clearly differentiated.

Berset and Caniaux (1983), working with parsley, found that measurements on the ground leaves gave better reproducibility than measurements on the whole leaves. The authors surmised that grinding the leaves released from interior leaf cells chlorophyll pigments which were of a different type and more highly pigmented than those in the surface cells. It was shown that the hue function correlated highly with extracted chlorophyll *a* and *b* content. These workers also suggested that their colorimetric procedures could be utilised in the quality control of parsley drying and storage processes.

A novel approach to the preparation of flaky materials for colour measurement has been suggested by Grieder (1989). Working with herbs, he utilised a pulverisation procedure with sand. This aided the dispersion and trapping of oils, pigments and moisture expressed from the interior cells of the test materials during the grinding process.

An example of powder colour measurement for process validation is provided by Hans and Floros (1998). They modelled the colour change of potassium sorbate during a series of time-temperature varying trials. Statistically significant changes in both *L* and *a* values suggest that a potassium sorbate based system may prove a good indicator for processes, such as dry-heat sterilisation, which involve high-temperature heating. Mendes *et al.* (2001) also measured roast and ground robusta coffee. Their combination of sensory, instrumental and mathematical tools provided a means of predictive modelling for the optimisation both of processing and sensory attributes.

**Table 5.1** Colour measurement of powders, granules and flakes

Reference	Sample	Preparation	Temperature (°C)	Presentation (viewed from above/below)	Illumination/ aperture diameters (mm)	Standard	Instrument <sup>a</sup>	Measurement <sup>b</sup>
Johnston <i>et al.</i> (1980)	Durum semolina	Equilibrate to 15% H <sub>2</sub> O mill to semolina flour	NM	20 g samples	NM	NM	A(1)	<i>b</i>
Seakins (1971)	Casein	Sieve to 6 different particle ranges. Suspend samples in benzene	NM	0.5 cm light path	T <sup>c</sup>	Transmittance standard at 455 nm	B	% T at 455 nm
Little and MacKinney (1956)	Coffee beans at different roasts	Beans ground in commercial coffee grinder, sieved to 5 size ranges	NM	Ground coffee clear glass cell	NM	White tile neutral grey tile	C	Y
Berset and Caniaux(1983)	Dried parsley leaves	Leaves ground 1 min. in ball grinder. sieved to 80–250 µm particles	NM	NM	NM	NM	D	L*, a*, b* X, Y, Z, X, Y
Habib and Brown (1956)	Potato crisps	Crisps size reduced by milling 5 min. in Oster mill	NM	Viewing cell 89 mm high by 51 mm diameter. Sample depth of 76 mm (below)	NM/51	Ivory tile	E	L, a, b
Grieder (1989)	Herbs	Mix 5 g herbs with 5 g sand using automated mortar mill	20	Black metal cell with glass base, 55 mm diameter by 60 mm deep (below)	51/55	White tile	A	L, a, b, ΔE, ΔC
Hans and Floros (1998)	Potassium sorbate powder	Ground to a fine powder then baked	0	Glass cell	NM	NM	A(6)	L, a, b

<sup>a</sup>For instrument codes see Table 5.6; <sup>b</sup>For colour scales see Chapter 3; <sup>c</sup>Transmission cell; NM: not mentioned in the reference.

## 5.6 Particulate and lumpy solids (Table 5.2)

In order to eliminate problems associated with the differences in surface properties and shapes of commercially available dehydrated vermicelli, André and Pauli (1978) ground the samples down to powders, then formed tablets from the powders using a hydraulic press. The 40 mm diameter by 10 mm thick tablets could be measured directly on the head of the colorimeter. Good correlations were found between colour co-ordinate values and the  $\beta$ -carotene content derived from the egg component of the pastas. Pastas made from different flours could also be differentiated in terms of colour values.

Hazelnuts have been evaluated by Ozdemir and Devres (2000). They measured the colour after roasting of both the intact kernel and a ground hazelnut powder. Significant changes occurred in both  $L$  and  $a$  measured values with differences between whole and ground nut results suggesting that roasting level should not be monitored by considering either of these uniquely. By fitting the data to an Arrhenius type model, the authors established a generalised model describing colour changes as a function of temperature and time.

Fresh pasta colour has been measured at the author's laboratory (CB) (1988), the subject materials being spinach and egg varieties of ravioli, fettuccine, angel hair and tortellini. Samples were provided packed under gas atmosphere. It was found that food substances of this type required immediate measurement after removal from the package since surface colour changes started to occur as soon as the specimens were exposed to air. The effect was most noticeable with thinly cut angel hair. Samples were placed quickly into large area glass cells and measured at a large aperture instrument port within 30 seconds of removal from the package.

## 5.7 Large area solid foods (Table 5.3)

Sapers and Douglas (1987) measured enzymatic browning effects at cut surfaces of pears and apples, by cutting 22 mm diameter plugs from the samples using a cork borer and then placing the plugs on a glass cell over the instrument port. Browning could then be followed with time by measuring the  $L$  and  $a$  values.

A similar preparation technique has been utilised by Konstance *et al.* (1988) on beef chuck. In this case, samples were taken from 2.54 cm thick slices of chuck using a cork borer. The cylinders were then inserted in a previously bored rubber stopper placed on a glass cup. Components of the chuck meat such as lean, fat, bone and ligament could be separated in terms of  $\Delta E$  and colour values.

Eagerman *et al.* (1978) have followed the colour change with time in beef semimembranous muscle. Approximately 1 cm thick slices were placed against the port of a spectrophotometer and the reflectance spectra measured between 600 and 650 nm. The formation of a depression in the region of the spectral curve around 632 nm is an indicator of brown metmyoglobin pigment production.

**Table 5.2** Colour measurement of particulate and lumpy solids

Reference	Sample	Preparation	Temperature (°C)	Presentation (viewed from above/ below)	Illumination/ aperture diameters (mm)	Standard	Instrument <sup>a</sup>	Measurement <sup>b</sup>
Berghaller <i>et al.</i> (1983)	Potato cubes, dried	Commercially available cubes packed into cell to depth of 60 mm	NM	Dull black Al cell 60 mm internal diameter, height 60 mm, with optical glass base 1.2 mm. thick (below)	50/NM	White enamel tile	G	Y, L, a, b
Bardseth <i>et al.</i> (1988)	Dry sausage, salami type	Slices, 3mm thick	NM	No cover used, measured against black background	50/50 25/32	White tile	H(1) A(4)	L*, a*, b*
Lee <i>et al.</i> (1988)	Frozen green beans	Boiled 5 min. from frozen. Drained 1 min.	NM	10 cm square acrylic cell	Large/NM	White tile	A	L, a, b
André and Pauli (1978)	Dried vermicelli (of various shapes)	Pasta ground, sieved through 0.28 mesh, 10 g of powder formed to a tablet 40 mm diameter by 10 mm thick	NM	Faces of tablets measured directly on sensor port	NM/10	White enamel tile	G	X, Y, Z X, y
Miller and Burns (1971)	Peanut hulls (internal colour)	Peanuts harvested, shelled, and sorted subjectively into mature or immature categories	NM	Peanut hull halves directly on narrow beam sensor port	Narrow/NM	Porcelain coloured tile	I	Rd <sup>c</sup> , a, b
Authors' laboratory (1988)	Fresh pasta, angel hair and tortellini	Pasta portions packed into 89 mm diameter cell to cover optical glass base, flat against glass when possible	20/22	Reflectance through glass base. Black cover on cell (below)	50/89	White tile and black glass	I(3)	L, a, b
Ozdemir and Devres (2000)	Hazelnuts	Whole roasted kernel and ground roasted powder	NM	Direct onto kernel NM	NM	NM	H(3)	L, a, b

<sup>a</sup>For instrument codes see Table 5.6; <sup>b</sup>For colour scales see Chapter 3; <sup>c</sup>Luminosity value for some older instruments. Rd = Y; NM: not mentioned in the reference.

**Table 5.3** Colour measurement of large solids

Reference	Sample	Preparation	Temperature (°C)	Presentation (viewed from above/ below)	Illumination/ aperture diameters (mm)	Standard	Instrument <sup>a</sup>	Measurement <sup>b</sup>
Konstance <i>et al.</i> (1988)	Beef chuck	Sliced 2.54 mm thick	25	Glass cup (below)	NM/19	White plate	I(1)	$\Delta E, \Delta C, YI^c$
Eagerman <i>et al.</i> (1978)	Beef semimembranous muscle	Sliced $12 \times 8 \times 1$ mm	0	NM	NM	NM	K	R (632, 614 nm)
Freedman and Francis (1984)	Fruit jellies	Juice-sucrose-pectin jellies	NM	$4 \times 5$ mm transmission cell, 2 cm light path	T	Distilled water	A(5)	$L, \theta$
Nagle <i>et al.</i> (1979); Reeves (1987)	Whole red peppers	Blanched whole fruit	NM	Measured around the shoulder of the fruit	NM	Colour plate	I(4)	$L, \theta$
Shewfelt <i>et al.</i> (1989)	Fresh tomatoes	Whole, measured 'as is'	NM	Measured at 8 equidistant locations on surface of fruit	NM	Pink tile	I(6)	$L, a, b, \theta$ , chroma
Lin <i>et al.</i> (1989)	Apples, fresh	Whole, measured 'as is'	NM	Measured at 8 equidistant locations on surface of fruit	NM	Pink tile	I(6)	$L, a, b, \theta$
Sapers and Douglas (1987)	Raw apples and pears	Plug (22 mm diameter) removed from halved fruit. Transverse cut across plug just prior to measurement	20	Fresh transverse surface of plug centred over instrument aperture	Large/19	White tile	I(1)	$L, a, b, X, Y, Z$
Beardseth <i>et al.</i> (1988)	Potatoes, raw	Cut lengthwise, covered with polyethylene film	NM	Readings taken immediately through poly film	8/8 50/50 25/32	White tile	H(2) H(1) A(4)	$L^*$
Beardseth <i>et al.</i> (1988)	Raw cauliflower	Cauliflower was measured 'as is' directly at port	NM	No cover used	50/50 25/32	White tile	H(1) A(4)	$L^*, a^*, b^*$
Hetherington <i>et al.</i> (1990)	Fresh tomatoes	Directly on tomato equator	12		NM/20	White tile NM	M	$L^*, a^*, b^*$ , XYZ, $L^*, a^*, b^*$

<sup>a</sup> For instrument codes see Table 5.6 ; <sup>b</sup>For colour scales see Chapter 3; <sup>c</sup> Yellowness index  $YI = 100/Y$  (1.277 X – 1.06Z) NM: not mentioned in the reference. T: transmission cell.

Measurements on whole fruits have been carried out by Nagle *et al.* (1979) and Reeves (1987) for capsicums, Shewfelt *et al.* (1989) for fresh tomatoes and Lin *et al.* (1989) for apples. In all cases the fruits were measured directly on the instruments, taking a number of readings around the circumference of each specimen. High correlations were obtained between colour values of the whole fruit and extracted xanthophyll pigment in red peppers. Shewfelt *et al.* (1989) showed that for whole tomatoes there was a significant difference between colour values due to cultivar, storage treatment and evaluation period. Simulated warehouse storage conditions were found to delay ripening colour changes.

A useful technique for raw fresh cut potatoes has been employed by Baardseth *et al.* (1988). The pieces were wrapped in polyethylene film to retard browning and surface drying before placing on the instrument port; slices were then measured immediately. The  $L^*$  values were compared between two instruments. Significant correlations were obtained between results from the Minolta large measuring area instrument (CR-110) and the HunterLab large measuring area instrument, as well as between the small area Minolta (CR-100) and the HunterLab instrument.

Hetherington *et al.* (1990) made tristimulus colour measurements on whole tomatoes. Using  $a^*$  data they obtained significant correlation with both a mechanical measure of ripeness (puncture test) and with sensory observations associated with colour changes from green to red. By sectioning the fruit samples (2mm thickness) and then measuring reflectance data over white and black backgrounds, absorption and scattering coefficients based on Kubelka-Munk theory were also obtained. These provided the necessary link, when combined with colorimetric data, to quantify the aspect of translucency. Ripeness, as used as a harvesting/buying criterion, requires both visual and tactile assessment and so by combining colour, mechanical and sensory testing this study clearly shows the potential for development of on-vine quality control instrumentation.

## 5.8 Pastes and slurries (Table 5.4)

This group of materials offers the most problems in colour measurement terms, because of the influence of translucency and light trapping effects on the colour results. Baardseth *et al.* (1988) reported that the prediction index for yam colour measurements was the least precise in the red/green parameter than for all the other foods evaluated in the paper, such as mashed potato, raw potato and raw cauliflower. The authors suggested that homogeneity was a key factor in obtaining the most precise prediction index, and that in the case of yams, therefore, variations in the fruit pieces may have been a factor in the loss of precision.

Nagle *et al.* (1979) homogenised pepper pods, the seeds and stems having been removed, prior to introduction of the sample into a cup provided with a white backing. Care was taken to allow the purées to settle; they were then

**Table 5.4** Colour measurement of pastes, purées and slurries

Reference	Sample	Preparation	Temperature (°C)	Presentation (viewed from above/ below)	Illumination/ aperture diameters (mm)	Standard	Instrument <sup>a</sup>	Measure- ment <sup>b</sup>
Baardseth <i>et al.</i> (1988)	Mashed potatoes and yams	Commercially available samples	NM	Plexiglass cell, 10cm cube with 3 mm quartz glass front	50/50 25/32	White tile	H(1) A(4)	$L^*$ , $a^*$ , $b^*$
Nagle <i>et al.</i> (1979); Red pepper Reeves (1987)	Red pepper purée	Milled whole fruit	NM	Sample cell with white top plate (below)	NM	Colour plate	I(4)	$L$ , $a$ , $b$
Francis (1985)	Blueberry toppings	Purée blueberries incorporated in topping mix with 15% or 30% fruit content. Formulations pasteurised	NM	3 cm depth in 6 cm diameter cell (below)	NM	White tile	I(1)	$L$ , $\theta$
Clydesdale and Francis (1969)	Spinach purée	Milled spinach processed to F <sub>0</sub> 4.9	NM	NM	NM	Grey tile, pressed Ba <sub>2</sub> SO <sub>4</sub>	A C	$X$ , $Y$ , $Z$ $L$ , $a$ , $b$
Huang <i>et al.</i> (1970)	Squash purée	Commercially available samples	NM	6.4 cm diameter cells, sample thick- ness between 2 and 8 mm, backed by black or white papers	14/47 47/47	Pressed Ba <sub>2</sub> SO <sub>4</sub> pink tile	L L A	$G$ , $R$ , $B$ $X$ , $Y$ , $Z$
Silva <i>et al.</i> (1989)	Sweet potato mash	Various potato sizes were canned or steamed, then frozen or canned	NM	NM	NM	Orange tile	A	$L$ , $a$ , $b$ $\theta$ , $\Delta C$
Bosset <i>et al.</i> (1986)	Yoghurt	Commercially available samples	NM	500 ml measuring cell, yoghurts stirred before measurement	NM	NM	F(1)	$L$ , $a$ , $b$
Brimelow (1987)	Tomato paste	Dilute to 12% TSS <sup>c</sup>	20	50 mm depth in 62.5 mm diameter cell (below). Black cover	51/51	Red tile	A(2)	$a/b$

<sup>a</sup>For instrument codes see Table 5.6; <sup>b</sup>For colour scales see Chapter 3; <sup>c</sup>TSS: total soluble solids (%) measured on the sugar scale; NM: not mentioned in the reference.

vibrated to remove air bubbles. Good correlations were obtained between colour values and both xanthophyll and total pigment concentrations.

Squash purées have been measured by Huang *et al.* (1970) using reflectance techniques in conjunction with the Kubelka-Munk concept. In this concept, reflectance measurements of thin layers of material are made over both white and black backgrounds. A reflectance value at infinite thickness can be calculated as well as ratio values for light scattering and absorption. In the work on squash purées, a tristimulus colorimeter was employed previously 'hitched' to a pink standard. Good correlations were achieved between visual ratings and colour values, and between visual ratings and Kubelka-Munk absorption/scattering ratios.

This idea of 'hitching' was further used by Silva *et al.* (1989) who examined sweet potatoes prepared by various methods. All samples were mashed or puréed for chemical and physical analyses. The authors utilised an orange colour tile for 'hitching' standardisation of a Hunter D25 instrument. Colour differentiation of the process variables in the  $L$ ,  $a$ , hue angle and saturation or chroma measurements could be used, the authors suggested, in predicting the quality of these types of products. The lower  $L$ ,  $a$  and saturation values of canned samples versus their frozen equivalents were indicative of the more extensive heating which the canned products received.

In an extensive study to evaluate the effects of packaging materials, light and storage on solid, whole milk yoghurt, Bosset *et al.* (1986) measured colour changes in the yoghurt in a specially designed 500 ml capacity cell for the MacBeth Spectrophotocolorimeter. Their results indicated that changes in the  $L$ ,  $a$  and  $b$  colour values could be related to exposure to light through the various packages. In particular, the authors reported that Hunter  $a$ ,  $b$  values were sensitive indicators of product changes due to photodegradation.

Finally, an exhaustive study of some of the variables affecting the measurement of tomato paste colour has been carried out as part of the COST 90 programme by Brimelow (1987). The effects of such factors as paste dilution, test duration, instrument aperture size and illumination area, sample temperature, standardisation hitching-post tile temperature, and the presence or absence of extraneous light were all examined. A standard colour measurement procedure was advocated for this important food commodity. As a parallel effort in this work, a new European standard tomato red tile was specified by the COST 90 colour group participants, to act as a hitching post in the measurement of tomato pastes and purées.

## 5.9 Liquids (Table 5.5)

Many clear or lightly translucent liquids are measured by transmission or transreflectance techniques, as already discussed. The study of Skrede (1985) illustrates how colour measurement can be used to predict the shelf-life of a particular food material. Commercial blackcurrant syrups were packed in



**Table 5.5** Colour measurement of liquids

Reference	Sample	Preparation	Temperature (°C)	Presentation (viewed from above/ below)	Illumination/ aperture diameters (mm)	Standard	Instrument <sup>a</sup>	Measurement <sup>b</sup>
Francis (1985)	Blueberry beverages	Purée blueberries incorporated in beverage mix with 5% and 12% fruit con- tent. Formulation pasteurised	NM	2 cm transmission cell	T	Distilled water	I(2)	L, $\theta$
Francis and Clydesdale (1972)	Egg yolk	Homogenise	NM	20 mm depth glass (below)	10/NM	NM	A	a, b
Calvi and Francis (1978)	Carbonated grape beverage	Grape anthocyanins incorporated in carbonated beverage formulation	NM	1 cm transmission	T	Distilled water	I(5)	a/L
Skrede (1985)	Blackcurrant syrops	Dilution of the syrups 1 to 4 with H <sub>2</sub> O	NM	1 cm transmission cell	T	Water	J	L, a, b
Pilando <i>et al.</i> (1985)	Strawberry wine	Thawed berries, sugar and yeast fermented to wine, then racked and bottled with SO <sub>2</sub> wine stored in the dark	NM	Transmission in a 1.0 × 5.0 × 5.0 cm lucite cell, with gloss excluded	T	White tile	A(5)	L, a, b
Desarzens <i>et al.</i> (1983)	Liquid milk products	Cell filled with ca. 500 ml of liquid	NM	Reflectance in 500 ml sample cell	NM	White tile	F(1)	L, a, b
Cole and Roberts (1997)	Gelatine	Molten solutions	40	50 ml cuvette with a white tile cover	NM	Instrument white and black tiles	N	L, a, b
Author's laboratory(1999)	Liquid milk	UHT milk of varying fat content	25	1.2, 5, 10 mm white and black backing	5×10	White tile	F(2)	L*a*b*, $\Delta E^*$
MacDougall (1987)	Evaporated milk, Orange juice, Tomato paste	Dilution with distilled water to obtain various specified solids contents, giving opaque to translucent solutions	NM	Measuring cell containing over 5 cm depth. Black cover to 5	50 down to 10/50 down to 5	White tile	A(2)	L, a, b, y
Calvo <i>et al.</i> (2001)	Yoghurt	Base plain yoghurt plus added colour and flavour	7	30 mm thickness in a 65 mm diam. tray	13	NM	P	L*a*b*

<sup>a</sup>For instrument codes see Table 5.6; <sup>b</sup>For colour scales see Chapter 3; NM: not mentioned in the reference; T: transmission cell.

various packaging materials with different barrier properties. Storage lives varied between two months in high density polyethylene, 12 months in polyester and 18 months in glass. Combinations of Hunter  $L$ ,  $a$  and  $b$  values were found to give acceptable predictive abilities for visual colour.

Francis (1985) found in the blueberry beverages that an increase of blueberry content from 5 to 12 per cent did not result in a corresponding increase in visual colour. Since the berries are highly pigmented, he surmised that a much greater increase in pigment would be required to achieve an observable change in the beverage colour. Calvi and Francis (1978) were able to correlate the anthocyanins content of the extracted concord grape filter trim with the  $a/L$  index of grape beverages at various storage temperatures. Pilando *et al.* (1985) found that both anthocyanin content and Hunter  $L$  values were highly correlated with sensory panel judgements when assessing the colour quality of strawberry wine. Translucent liquids have been measured by Francis and Clydesdale (1972) (egg yolks), Desarzens *et al.* (1983) (liquid milk products), MacDougall (1987) (evaporated milk, orange juice) and others. These materials are measured by conventional reflectance techniques, ensuring usually that samples are at 'infinite' thickness.

Work in the author's laboratory (PJ) (1999) has used a path-length variability technique to get a quantitative measure of translucency in milk samples of varying fat content. By using cuvettes of systematically increasing thickness (1mm–10mm) and measuring over black and white backgrounds, samples could be characterised with the results comparing favourably to those obtained by sensory analysis. A similar study was carried out by Frost *et al.* (2001) who concluded that by combining thickener, whitener and cream aroma in a 0.1% fat milk, the sensory properties of a 1.3% fat milk could be mimicked.

Cole and Roberts (1997) used a different approach for their measurement of translucent molten gelatine solutions. Considering that the light which reaches the human eye during visual assessment of gelatine samples comprises both reflected and transmitted light, Cole and Roberts used a spectrophotometer and a transreflectance presentation of the samples. In such cases, it is essential that the scatter within the sample (in this case caused by molecular gelatine) is small in comparison to that from the white background tile. Tristimulus Hunter  $L$  data was found to correlate to colour values ascribed via Beer's law and a DCU index often used in the gelatine industry. The authors suggest that such a reference instrumental method of measurement could overcome many of the problems which occur as a results of the numerous 'in-house' colour grading systems currently in use. A similar transreflectance technique was used by Chantrapornchai *et al.* (2001) in their more fundamental investigation of the influence of flocculation effects on the optical properties of a model oil-in-water emulsion. Their results showed how colour measurement may be used to provide an increased understanding of how optical properties may have both processing and sensory relevance.

Francis and Clydesdale (1972) urged that egg yolks should be homogenised before colour measurement. This is because yolks are formed in a layered

**Table 5.6** Colour measurement instruments

<i>Code for Tables 5.1–5.5</i>	<i>Instrument</i>	<i>Geometry</i>	<i>Illuminant source</i>
A	HunterLab Color Difference Meter D25	45°/0°	C
(1)	D25A	45°/0°	C
(2)	D25M	Circumferential 45°/0°	C
(3)	DL25	Circumferential 45°/0°	NM
(4)	D25P	Integrating sphere 0°/d	C
(5)	D25 with transmission	Transmission	NM
(6)	D25-PC2	45°/0°	NM
B	Hilger Biochem	Transmission	NM
C	Colormaster Differential Colorimeter	Tristimulus reflectance	C
D	DU COLOR Color Difference Meter (Neotec)	Circumferential illumination	NM
E	Hunter Color and Color Difference Meter	Tristimulus reflectance	NM
F	Macbeth Spectrophotometer		
(1)	MS-2000	Integrating sphere 0°/d	C
(2)	ColorEye 7000	Integrating sphere d/8°	D65
G	MOMCOLOR D (MOM Hungarian Optical Works)	Tristimulus colorimeter	C
H	Minolta Chroma Meter		
(1)	CR-110	d/0°	C
(2)	CR-100	d/0°	C
(3)	II	Tristimulus colorimeter	NM
I	Gardner Automatic Color and Color Difference Meter	Tristimulus colorimeter	NM
(1)	XL-23	Tristimulus colorimeter	C
(2)	XL-23 with transmission	Transmission	NM
(3)	Colorguard 2000/45 sensor	Circumferential 45°/0°	C
(4)	XL-10A	NM	C
(5)	XL-10 with transmission	Transmission	NM
(6)	XL-845	Tristimulus colorimeter	NM
	Shimadzu 300-UV	Transmission	C
K	Bausch & Lomb Spectronic 20 with reflectance attachment	NM	NM
L	GE Recording Spectrophotometer	NM	NM
M	Instrumental Color System Micromatch 2000	d/8°	D65
N	BYK-Gardner Color-View Spectrophotometer	NM	NM
P	Hunter Labscan II	NM	D65

NM: not mentioned in the reference.

manner and poultry feed variations can, therefore, cause colour variations within each yolk. Desarzens *et al.* (1983) had a large capacity sample cell especially made to use with the MacBeth MS-2000 for liquid milk products. In a study of several types of liquid milk products, the authors were able to establish a relationship between measured colour changes in the products and a decrease of riboflavin content during storage exposure to light. MacDougall (1987) has shown that instrumental variables, such as aperture size and illumination area, have a large effect on the colour values obtained for a number of translucent liquids.

## 5.10 Conclusions and future trends

With advancing technology and decreasing hardware costs, colour reflectance measurement is becoming a much more accessible tool for the whole of the food industry. Using instrumental colour measurement in combination with sensory analysis and other physical measurements can provide insights into many phenomena associated with food manufacture. The ever-growing choice of instruments on the market means that the best purchase choice can be made depending upon the samples to be measured and the desired results. The variety of instruments, however, also increases measurement complexity and it is clear that to be able to compare colour measurements on different samples of the same food materials, either within a laboratory or between different laboratories, the experimental procedure must be carefully defined and standardised.

It is surprising how little information is given in much of the available literature on colour measurement, concerning the following essential areas:

- The choice of the viewing geometry and illumination type (which is associated with the selection of the instrument)
- The calibration of the instrument, including defining the calibration standards
- The selection of the instrument variables of aperture and illumination areas
- The selection, preparation and presentation of the samples
- The defining of other relevant details of the test procedure, such as the temperature.

With careful consideration of all of the above it is possible to make meaningful, reproducible and accurate colour measurement on most types of food products.

The advent of on-line analysis will facilitate colour measurement as an important tool for quality control and process monitoring in the future. The industry is moving away from simply monitoring the end product and towards using colour measurement as a tool from product concept, through ingredient quality assurance, processing control and final quality control. This also means that measurement itself is no longer confined to the R&D or QC lab of the end-product manufacturer. Every link in the food chain can take advantage of this relatively simple and cheap analytical technique. The development of digital technology is also changing the nature of colour reflectance measurement –

armed with a simple digital camera and the appropriate software it is possible to custom design colour analysis for a specific application. A future challenge, for the colour industry as a whole, is to try to harmonise all these new and existing techniques.

### 5.11 Sources of further information and advice

There are many sources of information available to those interested in the colour measurement of foods. As well as the references mentioned in this chapter, certain key publications should be highlighted:

Hutchings, J. B., 1994, *Food colour and appearance*, Glasgow, UK, Blackie Academic and Professional.

Hunter, R. S. and Harold, R. W., 1987, *The measurement of appearance*, New York, USA, Wiley-Interscience.

McDonald, R., 1997, *Colour physics for industry*, Bradford, UK, Society of Dyers and Colourists.

Several international universities have departments or research groups studying colour measurement, often in relation to food applications. Examples include:

The University of Derby, UK. Colour and Imaging Institute <http://colour.derby.ac.uk/>

Rochester Institute of Technology, USA. Munsell Color Science Laboratory <http://www.cis.rit.edu/mcsl/>

The University of Leeds, UK. Department of Colour Chemistry <http://www.leeds.ac.uk/ccd/>

Finally, there exist many institutions, and of course, colour measurement companies who can provide both general and specific information in the field of colour measurement.

The Colour Group (Great Britain) <http://www.city.ac.uk/colourgroup/index.html>

The National Physics Laboratory, UK. <http://www.npl.co.uk>

Multinational colour measurement companies include Minolta, HunterLab, DataColor and GretagMacBeth.

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## 6

# Colour sorting for the bulk food industry

S. C. Bee and M. J. Honeywood, Sortex Ltd, London

## 6.1 Introduction

Good food can usually be distinguished from bad food by colour. This may appear to be an obvious statement, but the implications for the food industry are significant. Human perception of colour has proved very effective in determining food quality. Sorting of food products using the human eye and hand is still widely practised in regions where labour rates remain low. However, where the cost of labour has increased, so automated techniques have been introduced.

As a consequence of increasing consumer awareness of food hygiene, it has now become a basic prerequisite for all optical sorting machines to identify and remove all gross contaminants (glass, stones, insects, rotten product, extraneous vegetable matter, etc.). In addition, optical sorting provides a cosmetic enhancement to the product by removal of blemished, discoloured and misshapen product. Contemporary consumers are also demanding increased quality and in conjunction with this, a litigation culture has developed. Especially in recent years, tighter EU and American Food and Drug Administration requirements on food quality have been implemented. Food processors benefit from using automated systems for food sorting, since a machine can maintain greater levels of consistency than hand sorting and frequently offers reduced labour costs.<sup>1</sup> Food processors are able to provide a premium quality product at increased margins, allowing their competitive positioning to be strengthened.

## 6.2 The optical sorting machine

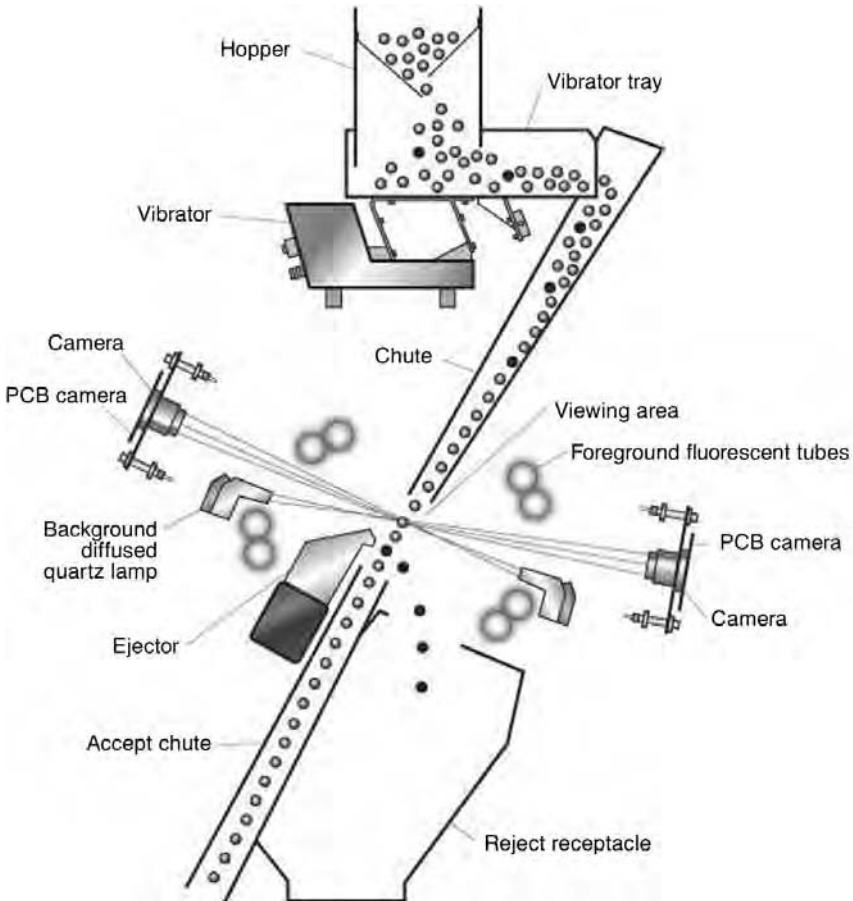
Colour sorters generally consist of four principal systems:

1. Feed system;
2. Optics;
3. Ejection process;
4. Image processing algorithms.

Figure 6.1 shows a typical layout for an optical sorting machine.

### 6.2.1 The feed system

In a bulk sorting system, dry products (rice, coffee, nuts) are fed from a vibrating hopper onto a flat, or channelled, gravity chute. To prevent excessive clumping,



**Fig. 6.1** Schematic layout of a typical optical sorting machine

fresh or frozen products are fed from a vibrating hopper onto an accelerating belt. Both methods separate the product into a uniform 'curtain', or monolayer. This ensures the product is then presented to the optical system at constant velocity.

### **6.2.2 The optical system**

The optical inspection system measures the reflectivity of each item. The inspection components are housed within an optical box and the objects under inspection travel either through, or past the optical box. Objects should not come into direct contact with any part of the optical box and are separated from it by toughened glass windows. The optical box contains one or more lenses and detector units, depending on the number of directions from which the product is viewed. Early optical-sorting machines viewed the product from only one side, which meant that they could only detect surface defects facing the optical system. Nowadays, two or three cameras are used to view the product from different angles as it leaves the end of the chute. Obviously, this increases the efficiency at which the system can identify defects. Lamp units, designed to provide even and consistent illumination of particles, are also usually contained within the optical box.

### **6.2.3 The ejection system**

The ejection system must be capable of physically removing unwanted product items from the main accept stream. The ejection process typically takes place while the product is in free fall; accept particles are allowed to continue along their normal trajectory, and rejects are deflected into a receptacle. Deflection is usually achieved by emitting short bursts of compressed air through nozzles aimed directly at the rejects, although large or heavy objects (e.g., whole potatoes) may require some sort of piston-operated device to mechanically deflect the rejects.

### **6.2.4 The image processing algorithms**

The image processing system classifies particles as either 'accept' or 'reject' on the basis of colour, or both colour and shape.

## **6.3 Assessment of objects for colour sorting**

The size, cost and complexity of sorting machines varies, depending on the size range of particles to be handled, the throughput requirement and the complexity of optical measurement. Machines are employed in sorting particles as small as mustard seeds; however, rice grains are among the smallest particles to be sorted on a large commercial scale. At the other end of



**Fig. 6.2** Sortex 90000 machine for rice, coffee, nuts and grains.

the size range are fresh and frozen vegetables (peas, green beans, cauliflower florets, etc.) and fruit and vegetables such as apples or potatoes. Seeds are usually sorted on a single- or double-chute machine at a throughput of 60, to 600 kg/hour. A higher throughput can be achieved on a multi-chute or a conveyor belt machine; rice at 16 tonnes/hour (320,000 objects/sec) and peas at up to 16 tonnes/hour are typical examples.

The products that can be handled by today's automatic sorting machines include seeds, coffee, rice, breakfast cereals, nuts and pulses; fresh, frozen and dehydrated vegetables; cherries (with and without stalks); olives; tomatoes; prawns; biscuits and confectionery. Foreign material such as stones, sticks and organic matter can be removed, as well as objects with defects such as discoloration and damaged skin. Figures 6.2 and 6.3 show two typical sorting machines.

## 6.4 Spectrophotometry

To determine whether a particulate food product is suitable for colour sorting, and which type of sorting machine and optical configuration is most suitable, samples of both acceptable and unacceptable produce must be measured and assessed in the laboratory.

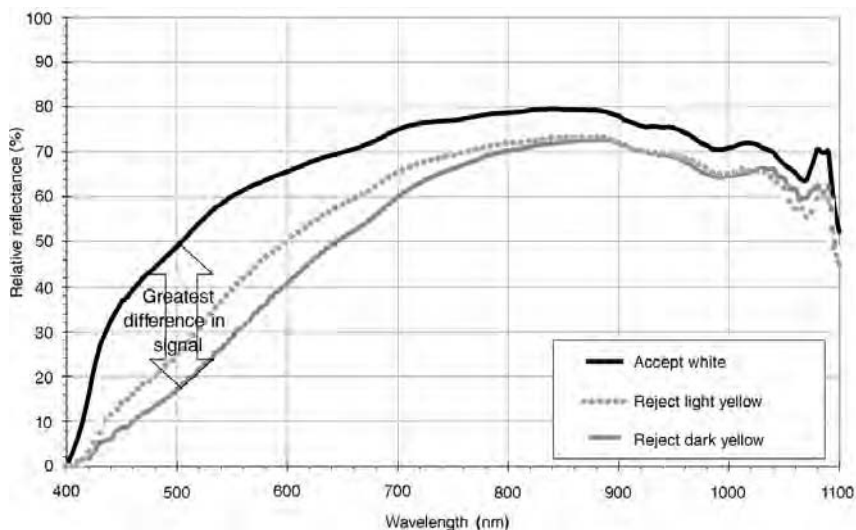
The term 'colour sorting' arises from the effect on the overall product appearance as a result of optical sorting. Unfortunately, the term is misleading.



**Fig. 6.3** Sortex Niagara machine sorting frozen peas.

The criterion the sorting machine measures when it inspects the product, is spectral reflectivity at particular wavelengths, rather than the colour as a whole. Figure 6.4 illustrates typical spectral curves obtained from white rice and white rice grains with yellow colour defects. The relative reflectance signal varies from black (zero and therefore no reflectance) to white (100% reflectance). The wavelengths cover the visible spectrum (400 to 700 nm) and extend into the near infra-red (700 to 1100 nm). Optical sorting exploits the region of the spectrum where the reflectance values for all acceptable product are either higher or lower than values for all unacceptable material. If this feature is present, then with the aid of band-pass optical filters, this part of the spectrum can be used as a basis for optical sorting.

Conventional spectrophotometry involves the measurement of carefully prepared surfaces under controlled optical conditions and illumination. However, practical industrial, bulk-sorting machines must deal with naturally occurring surfaces, viewed under non-ideal illumination conditions. Computer-controlled reflection spectrophotometers are now widely available and enable measurement of the appropriate optical properties of naturally occurring surfaces. Diffuse spherical broadband lighting is used to uniformly illuminate the item under test. The reflected light is then passed through a computer-controlled scanning monochromator, which splits the light into its constituent wavelengths. The output is measured using a suitable detector and sent to the computer. When the equipment is appropriately calibrated, the results can be



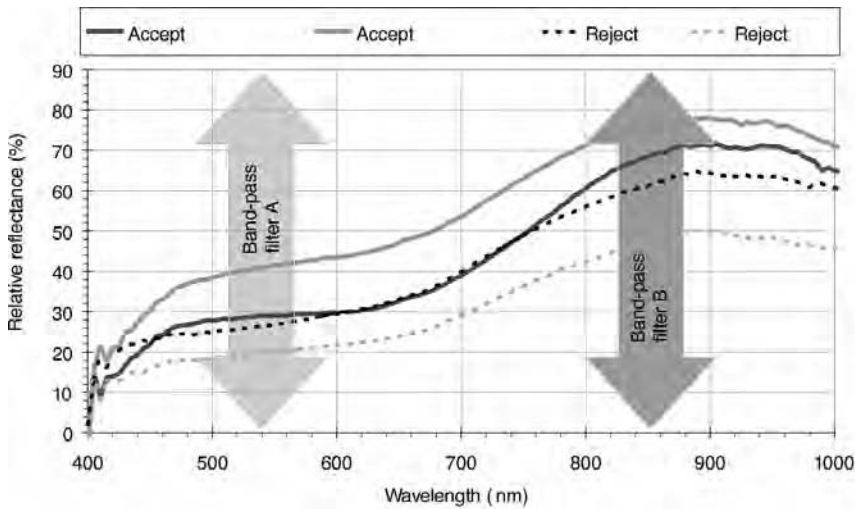
**Fig. 6.4** Visible reflectance spectra for white rice. In this example, a blue band-pass filter would be used for monochromatic sorting.

plotted showing the variation of reflectance (or transmission) with wavelength, for both acceptable and defective product.

## 6.5 Monochromatic and bichromatic sorting

Monochromatic sorting is based on the measurement of reflectance at a single isolated band of wavelengths. For optical sorting to be effective, there must be a distinct difference in reflectance within the selected waveband, between all the acceptable particles and all the reject particles (see Fig. 6.4). Removal of dark, rotten items from product like peanuts, or dried peas and removal of black peck from rice are typical applications of monochromatic sorting.

Sometimes it is not possible to find a single section of the reflectance spectrum where the intensity levels of accept and reject material are clearly separated. Therefore, it becomes necessary to compare simultaneous measurements at two different wavelength bands; a technique called bichromatic sorting. Figure 6.5 shows two sets of spectral reflectance curves obtained from green Arabica coffee. One set of data (solid lines) represents the lightest and darkest of acceptable beans, the other (dotted lines) represents the lightest and darkest of discoloured beans. In this case, no region of the spectrum allows successful separation of the two sets of curves. However, between 500 nm and 600 nm, the difference in the gradients of the two sets of curves is at its greatest. This is repeated between 800 nm and 900 nm. If measurement *A* is taken at 540 nm and measurement *B* is taken at 850 nm, the ratio *A:B* can be calculated. This ratiometric approach will yield a distinct signal difference between the 'accept'



**Fig. 6.5** Spectral curves for green Arabica coffee. Bichromatic sorting is necessary, since there is no one region of the spectrum where reject material can be successfully separated from the accept material.

and 'reject' reflectance spectra and allow effective optical sorting. (In principle, measurement A could be taken at 510 nm, but this would give a lower signal intensity.)

By measuring at two, rather than one region of band-pass wavelengths, bichromatic sorting involves twice as many optical components. At each optical inspection point, besides simply duplicating many of the optical components (e.g., filters, lenses), additional light-splitting devices and more complex signal processing are also required. Consequently, bichromatic sorting only is used when a simple monochromatic measurement is not adequate for effective optical sorting.

## 6.6 Dual monochromatic and trichromatic sorting

Dual monochromatic sorting is similar to bichromatic in that two wavebands are measured but instead of a ratiometric approach, the dual system sorts monochromatically in each of two separate wavebands. This type of sorting is used when it is necessary to reject two distinct types of defect or defects and foreign material, each of which exhibit different spectral characteristics. Dual monochromatic sorting is employed with white beans. Maize is rejected by detecting blue reflectance, and white stones are rejected using near infra-red. Some optical sorting applications require both monochromatic and bichromatic decisions to achieve a successful sort. Therefore, bulk optical sorting machines are available which are capable of making both types of measurement simultaneously.



Bichromatic sorting techniques can obviously be extended to trichromatic applications. The information gained from the third band-pass filter is often used for detection of gross defects such as the presence of foreign material such as: glass, stones, thistle heads, caterpillars, insects and mice! Trichromatic sorting almost always uses either green, red and infra-red band-pass wavelengths. It is unusual for the food industry to use the traditional machine vision community choice of red, green and blue.

Trichromatic sorting allows objects to be sorted according to their size or shape by suitable modifications to the sorting algorithm. In this way, objects of the same colour but different shapes can be separated. For example, pea pod can be distinguished from peas and green stalks or green caterpillars from green beans. Under- or over-sized objects along with mis-shaped objects with holes or cracks can also be detected and effectively removed. The Sortex Niagara machine is capable of simultaneously sorting for both colour and shape at a rate of 40,000 objects/second.

## **6.7 Fluorescence and infra-red techniques**

Of course, not all bad food is a different colour from good food. It has been found that certain non-visible defects (bacteria for example) fluoresce when irradiated with ultraviolet light (350 nm), and this property may be used as a basis for sorting. This technique was originally developed for removing 'stinkers' from green Arabica coffee beans, but has found applications in sorting peanuts, almonds and cranberries. However, the fluorescence effects can be short lived and may also depend on the circumstances and time elapsed since the product was harvested.

Over the last decade, the wavelength range used by sorting machines has been extended from the visible, further into the infra-red region. Here, both water absorption and other chemical effects play an important part in determining the reflectivity characteristics of food particles. Bichromatic infra-red machines are proving particularly effective in removing shell fragments from a variety of tree nuts.

## **6.8 Optical sorting with lasers**

Incorporating the use of lasers into bulk food sorting is a technique that is still in its relative infancy. A laser beam is used to illuminate the product and the reflected light is affected by the amount of laser light that is either scattered from the surface, or diffused within an object. Since the laser produces narrow beams of coherent light at a single wavelength, there is no need to use optical band pass filters. However, a disadvantage with this technique is in maintaining the high capacity demanded by the bulk sorting industry, in conjunction with the necessary resolution to accurately detect defects. The linear scan rate of the laser across the

width of the view and the velocity of the product determine the vertical resolution. To date, laser scanning is limited to approximately 2000 scans/second. For product travelling at 4 m/s, the vertical resolution is therefore of the order of 2 mm. By comparison, line scan CCD (Charged Coupled Device) technology offers around 5000 scans/second and a resolution of the order of 0.3mm.

Laser scanning also suffers from problems associated with the drop in illumination intensity. The signal-to-noise level is naturally compromised as a consequence of fanning out the laser beam across a line of sight. It can be quite a design challenge to reliably mechanically scan a laser by, say, a rotating polygon mirror, in the hostile temperature and debris-ridden environments that are often encountered in food-processing plants. To a limited extent, some successful sub-surface and texture inspection can be carried out on certain soft fruits and berries with laser light. In fact, the technique has already been commercially deployed for some product areas in the food industry, notably for dried fruits like raisins, or certain vegetables, nuts and tobacco. There is certainly scope for further study and possible wider exploitation of the technique.

## **6.9 The optical inspection system**

The range of wavelengths measured by an optical sorting machine is defined by the choice of light source, the properties of the optical filters (if used) and the properties of the detector itself. Similarly, at any particular wavelength, the characteristics of the electrical signal from the detector will also depend on these components. Once the optical characteristics of a product on which the basis for optical sorting have been identified and selected, the relevant wavelength bands must be isolated by selection of appropriate filters and illumination. A primary objective of selecting filters and lighting is to obtain the maximum possible signal-to-noise ratio from the detector at the required wavelengths, and the minimum possible signal at all other wavelengths.

## **6.10 Illumination**

When dealing with irregularly shaped objects, uniform, diffuse illumination is required to minimise highlights and shadows, since these would obviously detract from the measurement of true surface reflectivity. To eliminate shadows and highlights at the point of measurement, the particle should be surrounded by a spherical surface of uniform brightness. However, in practice this is just not possible due to the following constraints:

1. To allow a path for particles through the optical inspection chamber, there must be entry and exit points.
2. The position of the optical components will result in areas of different brightness, compared with the main chamber wall.

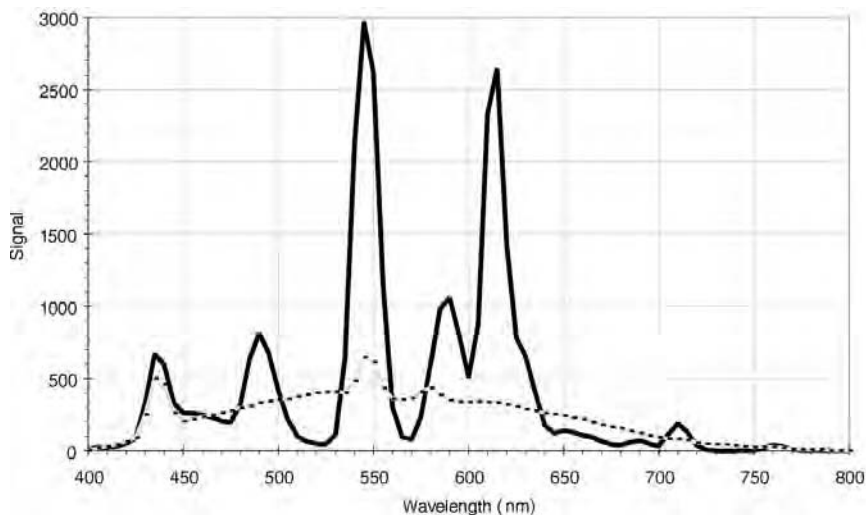
3. The use of light sources of finite size leads to non-uniform illumination.

Specular reflection is almost always a problem, even with a perfect diffuse illumination sphere. If a particle with a diffuse reflective surface is placed in such a sphere, then its true colour will be observed. However, if the particle surface is not diffuse, specular reflection will occur, giving highlights which do not exhibit the true colour of the surface. Clearly the highlights can adversely affect the optical system and consequently result in the incorrect classification of a particle.

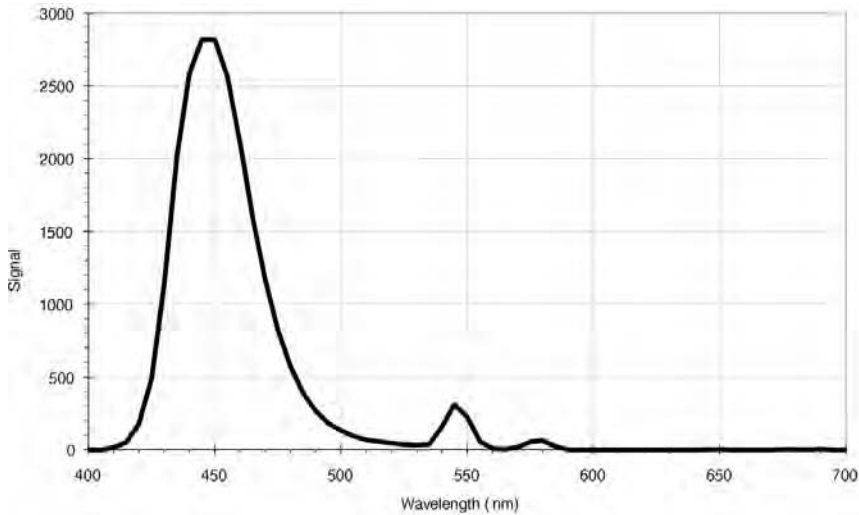
The most cost-effective form of illumination uses fluorescent tubes and/or incandescent filament bulbs. A number of lamps are arranged to provide as uniform a distribution of light as possible. With discrete lamps, diffusing windows are used in front of the bulb to diffuse the high-intensity point source of light emitted by the filament. To overcome some of the inefficient heat loss issues associated with incandescent lamps, arrangements using glass rods in conjunction with reflecting ellipses can be implemented.

Fluorescent tubes can be manufactured with different spectral characteristics depending on the phosphors used. The spectral range of fluorescent tubes spans the ultraviolet to the far visible red (Figs. 6.6, 6.7 and 6.8). The advantages of the fluorescent tube are its long life, diffuse light, low cost and relatively cool operation. Its disadvantages are that it is limited to the visible and UV wavelengths and requires a special power supply to prevent flicker.

The advantages of incandescent lamps are their inherent broad spectral range, from blue to the near infra-red and their D.C. operation. However, they suffer from being point sources that dissipate large amounts of heat. A typical emission

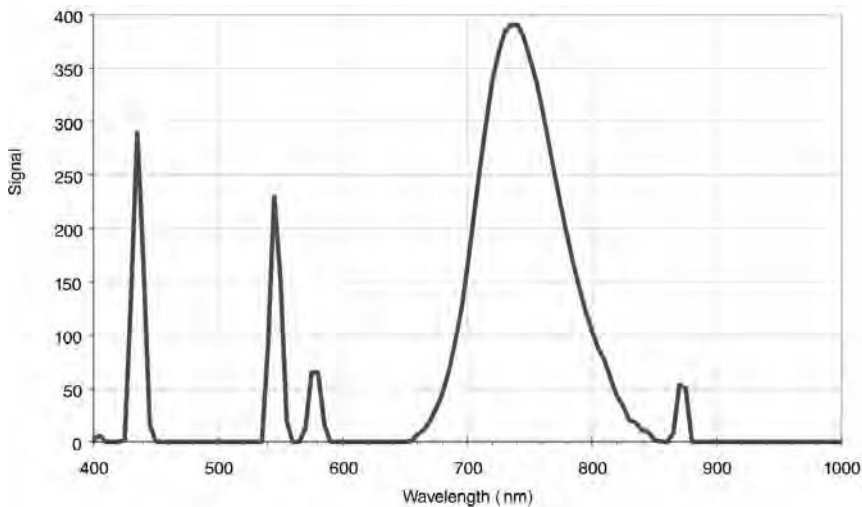


**Fig. 6.6** Emission spectra for two broad-band fluorescent tubes. Both are attempting to simulate pure sunlight, or natural daylight, where all wavelengths of the visible spectrum are present.

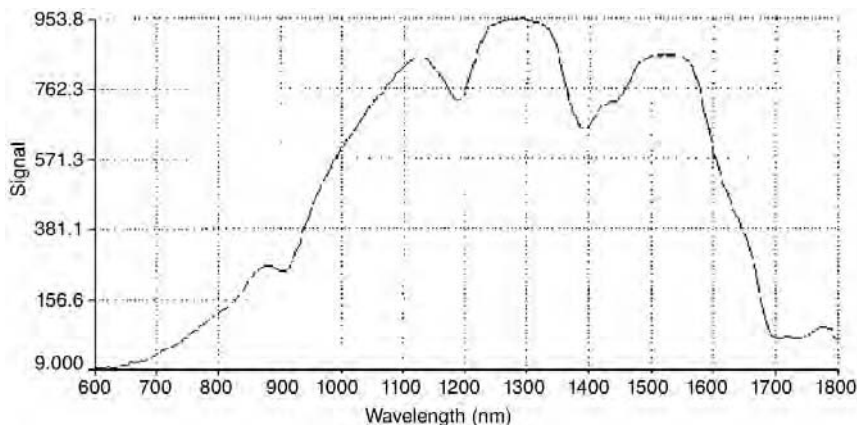


**Fig. 6.7** The emission spectrum from a fluorescent tube where special phosphors have been used to enhance emission in the blue region (450 to 500 nm) of the spectrum and suppress emission in the red regions (650 to 700 nm).

spectrum is shown in Fig. 6.9. In general, for optical sorting applications, fluorescent tubes are the most widely used, except in cases where near infra-red or infra-red measurements are required. The wider spectral range required for bichromatic machines necessitates the use of incandescent lamps, often in combination with fluorescent lamps.



**Fig. 6.8** Different phosphors can also be used to enhance the emission in the red region of the visible spectrum.



**Fig. 6.9** A typical broad-band emission spectrum from an incandescent bulb.

## 6.11 Background and aperture

The simplest form of inspection system views the particles through a small aperture and against an illuminated background. The brightness of the background is adjusted so that the optical system measures the same average value, with or without product. This is known as a ‘matched’ background because it matches the average brightness of product, including any defects. For effective shape sorting, where the boundary of each object must be apparent, an ‘unmatched’ background is used.

Matching the background offers an advantage in that measurement of reflectance is independent of object size, for example, in the case of a stream of particles containing reject items that are darker than accept items. With a matched background, whenever a dark defect passes the aperture there will be a decrease in signal amplitude and with a light particle there will be an increase in amplitude. Hence an unequivocal decision can be made by the electronics. However, if the background is lighter than the average of the product, then all product items would give a decrease in signal. In particular, small dark defects would give signals identical to those of large light particles and the two could not be easily distinguished. The intensity of the light reflected from a particle via the aperture is the product of the size of the particle and its reflectivity, including any area of discoloration.

The background usually consists of an array of suitably located lamps (or LEDs – Light Emitting Diodes) behind an optical diffusing material. In some cases a white, diffuse reflecting plate is used to reflect light from rear-mounted lamps or LEDs towards the detection optics. The aperture is usually a rectangular slit. The width of the slit must be sufficient to allow for scatter in the trajectories of the particles and for the range of anticipated object sizes. The height of the slit is maintained at a minimum, although allowing for sufficient signal to provide maximum signal to noise for detection, in conjunction with

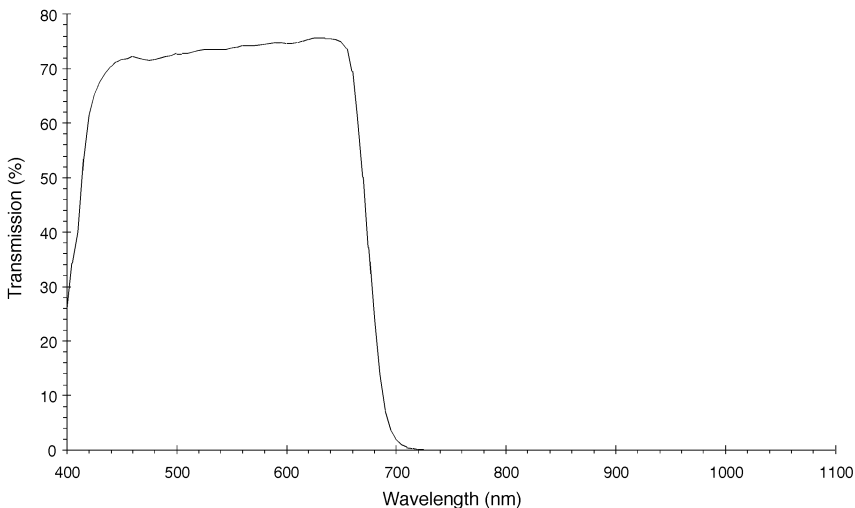
resolution and accurate timing of the delay between detection of a defect and rejection of a particle.

## 6.12 Optical filters and detectors

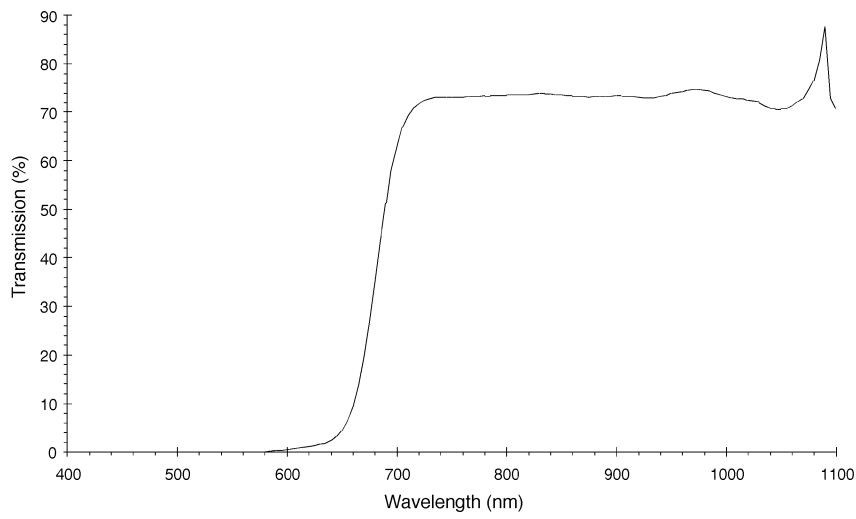
An optical filter is essentially a piece of coloured glass. An extensive range of optical filters are readily available as off-the-shelf components. Alternatively, custom filters can be made at a higher cost. Four basic types of filter are used for optical sorting applications:

1. Low pass: transmitting only below a certain wavelength (Fig. 6.10);
2. High pass: transmitting only above a certain wavelength (Fig. 6.11);
3. Band-pass: transmitting only within a band of wavelengths (Fig. 6.12);
4. Combinations of the above in a single filter e.g., a double band-pass filter (Fig. 6.13).

Prior to the advent of solid-state detectors, the photomultiplier tube was the best detector of visible radiation. The photomultiplier has a good signal-to-noise ratio that allows detection at low light levels, and a satisfactory response in the violet-blue (400 to 500 nm) region of the electromagnetic spectrum. However, due to their fragile mechanical construction, photomultipliers are not very robust. They also suffer from limited life, high operating voltage and poor deep red and near infra-red response (650+ nm). Following photomultiplier tube technology, solid-state technology now dominates the optical sorting industry. Initially the photodiode was used due to its comparative cheapness, mechanical robustness and almost indefinite life. However, compared to photomultipliers, photodiodes have a poor blue response.

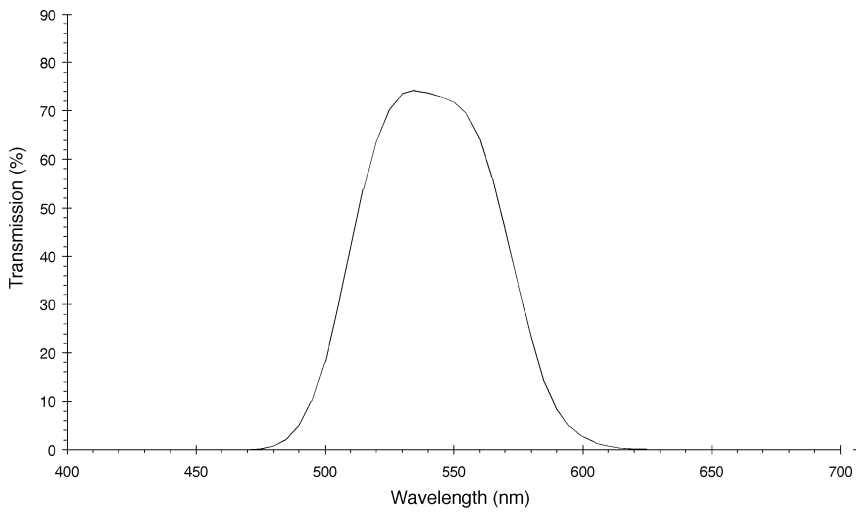


**Fig. 6.10** The transmission spectrum for a low-pass optical filter.

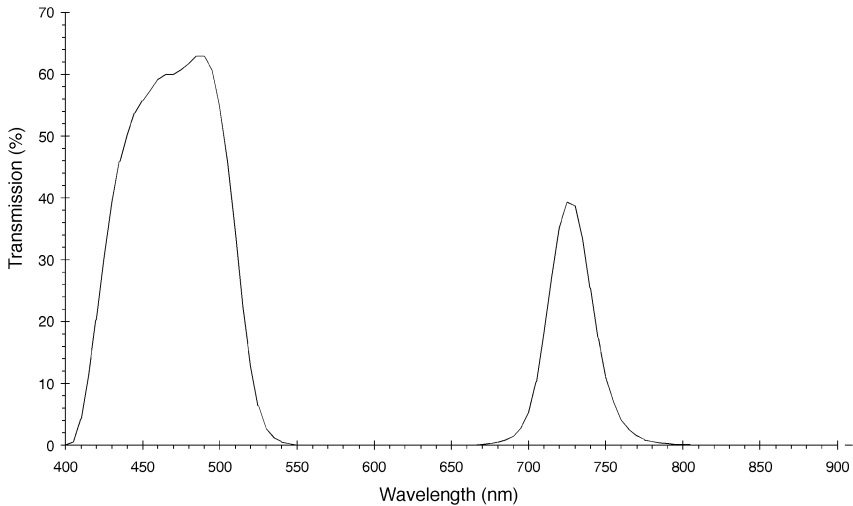


**Fig. 6.11** The transmission spectrum for a high-pass optical filter.

Contemporary optical sorting machines now employ high-speed line scan CCD (Charge Coupled Device) technology. Silicon CCD technology offers the advantages of high sensitivity, good broad band response (400 to 1000 nm), high spatial resolution and good quantum efficiency. Although CCDs are analogue sensors, their output is easily converted to digital form. Consequently, state-of-the-art, low-noise, high-speed digital processors can be used for subsequent signal processing. Unfortunately, CCDs continue to suffer from relatively poor response in the blue region.



**Fig. 6.12** The transmission spectrum for a band-pass optical filter.



**Fig. 6.13** A double band-pass filter.

In order to extend the detection range into the infra-red domain, other detector materials besides silicon are used. Having a good infra-red response (up to approximately 1700 nm), germanium detectors are readily exploited by the food-sorting industry for detection of foreign matter. Historically single photodiodes were used, however, germanium linear arrays are now commonplace and generally used in combination with silicon CCD technology. In fact, much of the infra-red technology developed for the telecommunications industry is now readily exploited for optical sorting applications.

### 6.13 The sorting system: feed

The product feeding system in a sorting machine should provide three basic functions:

1. Metering: to ensure that the optimum number of objects per unit time are fed through the optical inspection area.
2. Acceleration to a constant velocity: the time taken for objects to travel from the optical inspection point to the ejection point must be constant so that activation of the ejector can be accurately synchronised with the position of the object. Typically, the velocity of the product is of the order of 4 m/s. The delay between detection and ejection is between 0.5 and 100 ms.
3. Alignment: to ensure a controlled trajectory through the inspection and ejection points.

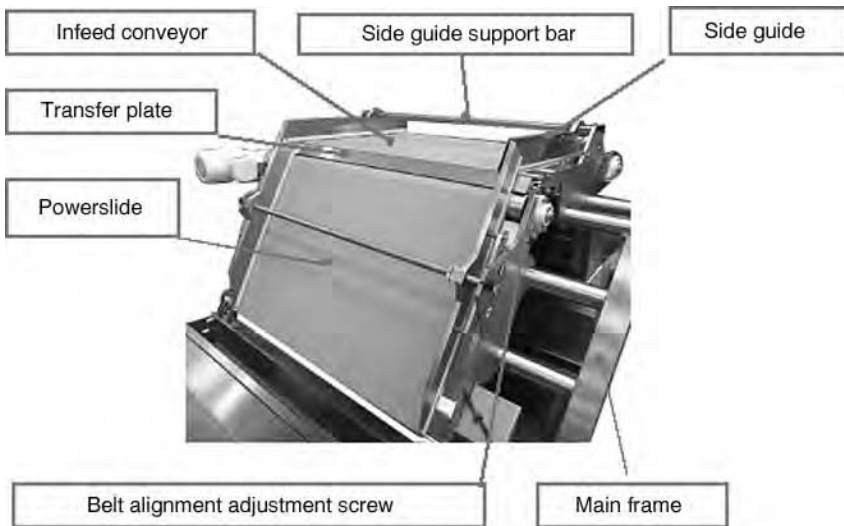
In reality, metering of product is achieved by a vibrating feeder tray, situated just below the output of a hopper. The following feed systems are commonly employed:



- an inclined gravity chute
- a flat belt
- an inclined belt (unique to Sortex)
- a 'C'-shaped belt
- contra-rotating rollers
- a narrow grooved belt.

To detect small blemishes on fruit and vegetables reliably, it is necessary to inspect the product from two sides. The traditional architecture of an optical sorting machine is to feed the product along a horizontal conveyor and then observe the product from top and bottom views as the product flies off the end of the conveyor. The drawback with this approach is that the bottom camera is soon covered in product. The Sortex Niagara machine overcomes this problem with the PowerSlide™ feed system (Fig. 6.14). The belt conveyor on the Niagara is inclined at 60° to the horizontal, such that the cameras can view the product from either side, and remain clean. This novel feed mechanism is known as the PowerSlide™ and is patented by Sortex.

The flat belt or gravity chute approach presents the product in a single layer, restricting the view to two sides, but allowing a much higher throughput of product to be achieved. In contrast, some feed methods channel and separate the particles into a single stream, each object dropping down after the other in 'single file' (see Fig. 6.15). This feed technique allows an all-round view of each object's surface, since three cameras can be positioned around the foot of the chute. Obviously three



**Fig. 6.14** The Niagara Powerslide™.



**Fig. 6.15** The Sortex 3400 machine is a compact design with two single channels. Three sensors surround each of the two channels providing all-round inspection and therefore a highly efficient sort. The 3400 is particularly suited to sorting low volumes of high-value commodities, since the yield and efficiency of the machine is very high.

views allow a very high-quality sort with an excellent yield. However, one disadvantage is the relatively low throughput of product compared to wide flat belt or chute techniques (a few hundreds of kg/hour, compared with several tonnes/hour). As a consequence, single-channel feeding is usually employed only for high-value products (Blue Mountain coffee, nuts like almonds or macadamia, selected beans and pulses, etc.). Throughput can be increased in single-channel feed systems by adding two or more channels to a machine.

### 6.14 The sorting system: ejection

The usual method for removing unwanted items from the main product stream is with a blast of compressed air from a high-speed solenoid or piezoelectric valve, connected to a strategically positioned nozzle. Pneumatic ejector valves must have rapid action, reliability, long lifetime (a minimum of one billion cycles) and mechanical strength. The fastest (a Sortex patented piezoelectric design) operates at a frequency of 1 kHz, firing a pulse of air for 1 to 3 msec. Ejectors operate at input pressures between 200 to 550 kPa (30 to 80 PSI), depending on

the size of the object to be removed. Typically, the ejection point is located outside the optical inspection area because the action of the air blast on a rejected object could cause dust particles and skin fragments to be blown around that could create false rejections. However, at the same time, it is advantageous to eject objects as soon as possible after the optical inspection point, due to unavoidable variations in the trajectory of each individual item.

The appropriate time delay between the inspection and ejection point is generated by electronic circuits. The accurate timing required to coincide the ejector air blast with that of the object to be ejected relies on the objects having constant velocity as they fall in front of the ejector nozzle. In practice, the tolerable variation in product velocity is about 5%. The trajectory of each particle also becomes harder to predict, the greater the distance between the viewing point and the ejection point. It can become a major design challenge to position the chute, optics and ejection system as close together as possible.

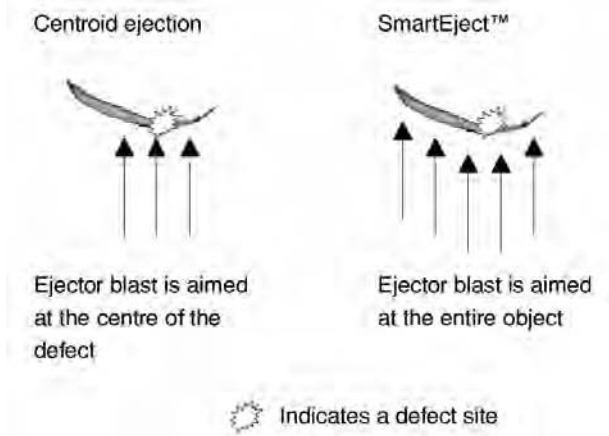
The operational lifetime of the ejectors must be in the region of at least a billion or more cycles. Food processing is usually a 24-hour-a-day, all-year-round operation. Operators cannot afford to shut down a machine regularly for even a few minutes to replace faulty ejectors. Under these circumstances, machine reliability and stability of operation are critical. For certain large or heavy objects a solenoid valve may be used to control a pneumatically operated flap or plunger to deflect rejected items. Specialised ejectors have been developed for pulps or slurries to remove rejects by suction and are mounted above a flat belt, downstream from the inspection unit.

#### **6.14.1 Smart ejection systems**

To accommodate long, or irregularly shaped objects, Sortex has developed the SmartEject™ system. With most optical sorting machines, the air blast is fired from one or more ejectors in an array, spanning the width of the belt or chute, just after the optical inspection area. The air blast is aimed solely at the centre of the defect (also known as ‘centroid ejection’). If the defect is a small blemish on a large object, then the blast may not be sufficient to remove the item. To combat this problem, the Sortex Niagara machine computes the location of the object encompassing the defect and fires the appropriate number of ejectors so as to fire at the entire object – this improved ejection system is known as SmartEject™ (Fig. 6.16). SmartEject™ fires one or more of 160 high-speed ejectors positioned across the line of view at the profile of an object, rather than at a defect, which improves both accept quality and yield.

#### **6.14.2 Three-way separation by two-way ejection**

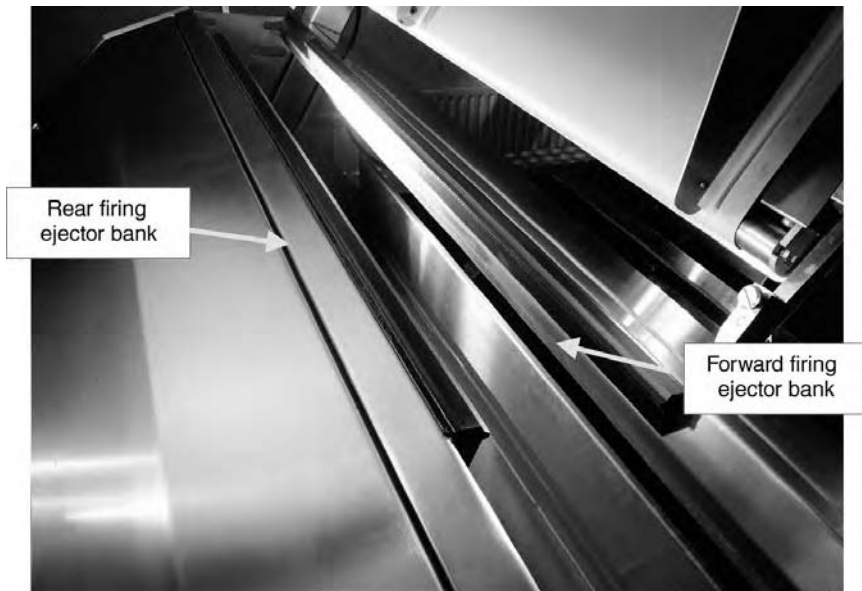
Sortex has also pioneered the ability to perform a three-way sort by adding a second bank of ejectors to the Niagara vegetable-sorting machine (Figs. 6.17 and 6.18). To date it has been the convention in optical food sorting to have only two-way separation, into accept and reject categories. The three-way separation



**Fig. 6.16** The SmartEject™ system for precise removal of larger objects with small defect sites that may not be located at the centre of the object.

by two-way ejection allows an additional product classification. For example, three-way separation for green beans now enables the following three categories:

1. accept
2. reject (rots, blemishes, foreign material, stalks. etc.)



**Fig. 6.17** Three-way separation by two-way ejection. Instead of a single rear-firing ejector bank, the Niagara is fitted with a second bank to fire in the forward direction.



**Fig. 6.18** Three-way separation by two-way ejection for green beans. From left to right: accept; accept with stalks; reject.

### 3. accept with stalks.

The advantage of the new third category is to allow recovery of otherwise 'good' product that would normally be rejected. Accept green beans with uncut stalks can be returned to the 'snibbers' (stalk cutters). In this way significant savings on recovered volumes can be made. Approximately 2% of green beans, typically running at 5 to 10 tonnes/hour are rejected on uncut stalks alone.

## 6.15 Cleaning and dust extraction

The successful application of optics in an industrial environment pervaded by dust, oil, starch, food debris or water poses major design issues for optical engineers. Considerable expertise is necessary to design an optical sorting machine capable of successful commercial operation under in-plant conditions. The operating temperature range encountered in a food processing plant varies between  $-5$  and  $+40^{\circ}\text{C}$ , making optical, mechanical and electrical tolerances critical to the effective operation of the machine. If the cameras of an optical sorting machine become obscured by debris, then the performance of the machine rapidly deteriorates. To protect the optical components from dirt or moisture, they are contained in an 'optical box' with a glass window. The position of this window in the optical path should be such that any small particles, that may settle on the surface are out of focus and therefore create minimum noise in the optical signal. However, it is essential that this window is kept as clean as possible and a number of facilities to achieve this may be provided on the machine.

Firstly, the product being fed to the machine should be as dry and dust free as possible. However, the action of storing it in a hopper and feeding it on a vibrating tray will usually create some dust. Hence, a dust extraction nozzle is often fitted at the end of the vibrating tray. In the case of a chute feed, the top of the chute may be perforated so that air can pervade the product stream to remove dust particles. In addition to dust extraction, the optical box window can be cleaned by means of compressed air jets. These 'air knives' as they are also sometimes called, provide a continuous curtain of air to prevent particles settling on the surface of the glass. If necessary, they can also provide a periodic high

pressure blast which removes any particles that may have settled on the window. Pneumatically driven blades or brushes can also be used to periodically wipe the window. In some machines this may be combined with an air 'blow-down' facility.

As a final precaution, any dirt created by the action of the ejector blast on the particles, may be drawn away from the window area by a dust extraction nozzle, positioned just below the optical box. Similarly, for wet or frozen product applications water jets and wiper blades can be substituted for air-based systems. Machines for sorting wet product are periodically hosed down with water, so must be water and dust proof to IP65 standards.<sup>2</sup> Similarly, dry product machines are also manually cleaned with an air hose. For hygiene reasons, all potential 'bug traps' must be designed out of all sorting machinery.

## 6.16 The electronic processing system

The electronic systems in sorting machines have progressed from the simple analogue circuits of the early machines to the advanced digital microprocessor-based circuits found in the present generation of machines. In contrast to many 'machine vision' applications, it is common for the optical data processing system of a bulk optical sorting machine to be hardware, rather than PC based. At the present time it is simply not practical to process 40,000 objects/sec for colour and shape followed by effective control of the ejection process with a PC-based system. Most of the setting up of the sorting parameters can be done by the machine itself, including in some cases the ability of the machine to 'learn' the differences between good and bad product. However, the operator is always given the opportunity to fine-tune the final result.

A sophisticated optical sorting machine will track the average colour of the product so that, even though the average product colour may change with time, the machine will continue to remove only the predefined abnormal particles. Optical sorting machines are often provided with a white calibration plate which is either manually or automatically placed in the optical view at user-defined intervals. The machine is then able to correct for any measurement drift that has occurred.

Once a machine has been set up for a particular product, all the machine settings can be stored in memory. This can be repeated for a number of different products and then, at a later time, the machine can be made ready to sort any of these products simply by recalling the appropriate settings from the memory. Alternatively, the settings can be used as a coarse starting point from which to fine tune a machine towards an optimum setting for a particular set of circumstances. Most food plants sort one particular product type. For example a rice mill may sort different varieties of rice, but would not, for example, suddenly switch to coffee. It would be unusual for a food processor to be sorting many different and diverse types of product.

Advanced sorting machines have a memory capability that can be exploited to provide information about the product for the operator. For example, the

number of rejects that have occurred in a certain time, or information about any drifts in colour in a certain batch of product. Information about how the machine itself is operating can also be provided to assist with preventative maintenance.

### 6.16.1 Optical detection and differentiation by shape

There are many applications in food sorting where the defects are similar in colour to the good product. For example: insect larvae in amongst blueberries take on the same colour as the berries; the stems on green beans are the same colour as the bean (Figs. 6.19 and 6.20); similarly pea and pea pod; or green caterpillars among green beans or peas. In order to be able to solve these types of applications, Sortex has pioneered the ability to sort objects on the basis of size, roundness, area, length and therefore, shape. In addition to colour and shape, the minimum size of the discoloration necessary for a particle to be rejected can also be defined.



**Fig. 6.19** The stems found in green beans with stalks present a major problem to freezers and canners. Niagara's stem recognition system is designed specifically to simultaneously remove stalks, along with foreign and extraneous material, typical defects such as black insect holes and beans outside user-specified lengths.



**Fig. 6.20** Advanced shape processing allows removal of many of the problems encountered in sliced carrots such as 'polos', ellipses (oblique slices), cracks and those with tangential mis-shapes. This is simultaneous with colour defect removal.

In the above examples, the larvae are elliptical in shape whereas the berries are round, and the stems on green beans are much thinner than the beans. A major innovation for the food-sorting industry has been the development of new vision algorithms for computing the size and shape of objects. Sortex has led the optical sorting industry in terms of the implementation of these algorithms in specialised electronic hardware. The Sortex Niagara machine's ability to sort objects on the basis of shape as well as colour, at high speed, is the basis of one of the major innovations of the machine. Up to 40,000 objects per second can be simultaneously sorted for shape and colour, across an 1100 mm wide line of view.

### 6.16.2 User interfaces

A typical machine will have either a keypad and a display unit (Fig. 6.21), or more commonly in contemporary machines, a touch-screen user interface (Fig. 6.22). A good user interface should allow the operator to set up and control the machine by means of an easy-to-follow series of menus. In addition, the display unit will provide the operator with information regarding the settings of the machine while it is sorting, together with details of any faults that may occur.

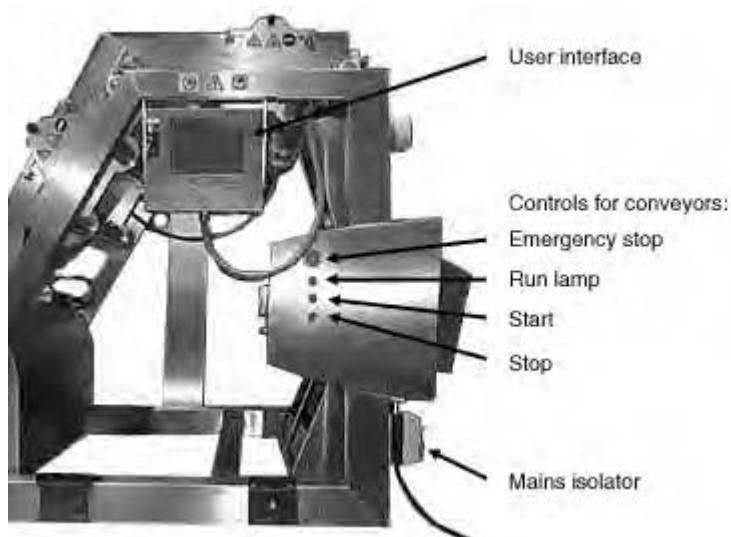
### 6.16.3 Mapping techniques

A bichromatic sorting machine using two band-pass filters, say green and red, makes a decision based on the ratio of the two signals in conjunction with the intensity of the individual signals. The situation can be represented as a two-dimensional 'colour' map by plotting the reflectivity of colour 1 versus that of colour 2 (Fig. 6.23). The bottom left-hand corner of this map represents the reflectivity from a black particle (0% reflectivity) and the top right-hand corner represents the reflectivity from a white particle (100% reflectivity). The boundary curve in Fig. 6.23 is the reflectivity map contour, outlining the acceptable product as seen by the sorting machine for a typical product. The contour line represents the chosen accept/reject threshold. The '+' within the map contour is the background 'balance point', that represents the average colour of the product.



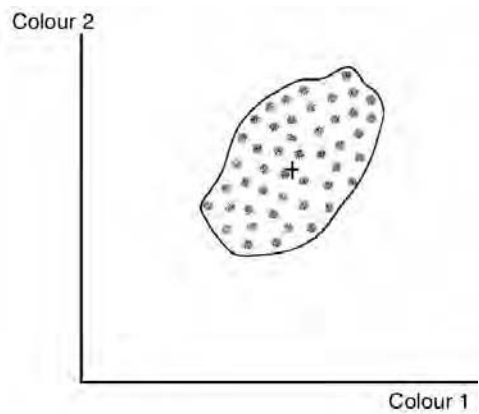
**Fig. 6.21** A user interface consisting of dot matrix alpha numeric text display and keypad.





**Fig. 6.22** Detail of the touch screen user interface and typical operator controls.

A major part of setting up an optical sorting machine is to achieve the best overall accept/reject ratio for the product being sorted. The operator can do this by using the user interface to adjust the shape and size of the map contour (Figs. 6.24 and 6.25) to match as accurately as possible the map contour of the product batch. The sorting sensitivity increases as the machine map contour is decreased in area as it approaches the area of the map contour of the product batch. Product within the area bounded by the threshold levels is accepted and product outside is rejected. These techniques allow an optical sorting machine to remove a far greater range of defects with greater accuracy and without the penalty of



**Fig. 6.23** A bichromatic colour map representing the distribution of colour 1 versus colour 2.

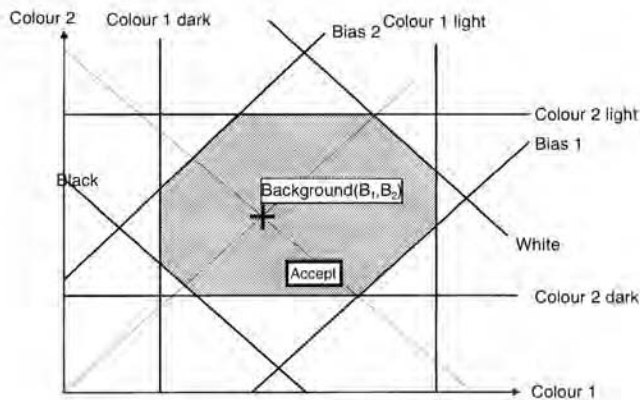


Fig 6.24 Bichromatic sensitivity thresholds.

removing large amounts of acceptable product. Obviously, these techniques can be extended into three dimensions for trichromatic colour sorting.

6.17 The limitations of colour sorting

There is often a misunderstanding that a colour sorter can remove 100% of the defects from a given batch of product. In practice this is impossible. A colour sorter will reduce the concentration of defective product, but it can never be 100% effective. All colour sorters are bound to remove some acceptable objects and fail to remove some of the defective objects. There are several reasons for this. Sometimes, the physical size or the colour difference of the defect from the product may be too small for accurate detection. Occasionally the machine may detect a defect and remove the object, but the object re-enters the accept stream after it has been ejected as a consequence of a random collision. Ejector

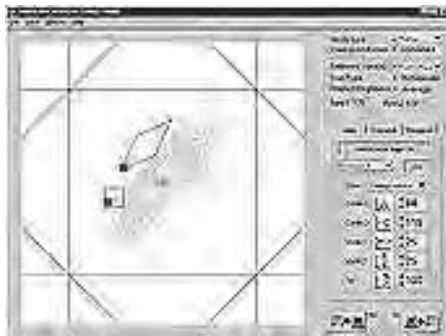


Fig. 6.25 A bichromatic colour map, as displayed on the user interface.

performance and minimal positional pitch of the ejectors in the array below the optical system can also become a limitation for accurate ejection. At present, the smallest ejectors have a 3 mm pitch. This limits the ejector 'resolution', especially for small products like rice or sesame seeds.

Machines can be adjusted by operators to optimise their performance. Sensitivity is one of the principal parameters that the operator can change. Increasing the sensitivity will result in the machine rejecting more defective material. However, a greater proportion of good product will also be rejected as the sensitivity threshold approaches the average product colour. There is normally a compromise point between achieving a high sorting efficiency and optimum yield (the ratio of good to bad material that is rejected). This compromise point is primarily achieved as a result of operator experience and training.

There are physical limits to the product throughput that a sorting machine can successfully achieve. If the product flow is increased above the upper limit, the product sheet will no longer be a monolayer. Objects will overlap and sorting performance will deteriorate, since many defects will be obscured and therefore will not be detected by the optical system. Increasing the flow of product through the machine will also result in increased good product being lost, since overlapping and colliding products are difficult to eject efficiently. Table 6.1 illustrates some typical performance figures for a variety of products sorted on different machines. The throughputs are quoted in ranges, since the throughput increases as the level of input contamination decreases.

## 6.18 Future trends

Computer vision systems are increasingly being used in general manufacturing, for example in pick and place applications such as printed circuit board (PCB) population and manufacture. However, the demands of the food industry are generally far greater. At present, there is only a limited range of computer vision equipment available for use in the food industry. However, in the future this is likely to change. Two factors are limiting the rate at which computer vision systems are being introduced to the food industry:

1. The data-processing rates required in a sorting machine for the bulk food processing industry are very much higher than those in a similar inspection machine for manufactured objects.
2. The development of improved materials handling and separation systems is not keeping pace with the dramatic advances being made in computer hardware technology.

A computer vision system potentially offers many benefits over a conventional colour sorter. The ability to simultaneously sort objects on the basis of several different criteria would be a primary advantage. However, for the immediate future, the most likely application of advances in electronic hardware is the gradual improvement in performance of the present generation of sorting machines.

**Table 6.1**

Product	Machine	Sorting criteria	Throughput (tonnes/hour) per machine
Whole green beans	Niagara – Trichromatic colour sorter, 1 m wide belt	Remove attached stems and blemishes	8
Green coffee	Truechromatic – 48 channel, gravity chute bichromatic sorter	Remove defective beans and foreign material	3 to 6
Parboiled rice	90004 Focus – 128 channel, 4 gravity chute, monochromatic sorting machine	Remove spotted, discoloured rice and foreign material (stones, glass, paddy, etc.)	5 to 10
Frozen peas	Niagara – Trichromatic colour sorter, 1 m wide belt	Remove foreign material, pea pod, sticks, etc. by colour and shape.	10 to 16

The optical sorting industry readily exploits advances in components, manufacturing processes and designs. At the present time, defect detection is mostly carried out in the visible and near infra-red wavelengths, mainly because of the added cost of infra-red detector technology. However, other wavelengths are already used in other areas of the food-processing industry. X-ray techniques are often employed as a final check for foreign material in packed or processed foods, or to detect hollow potatoes, for instance. Ultraviolet light can be used in some nut-sorting applications, especially to detect fungal-infection sites that fluoresce when exposed to UV light. The natural progression of monochromatic to bichromatic sorting will inevitably lead towards wider use of trichromatic technology. As the cost of lasers continues to decrease, so laser technology may become commonplace for texture or sub-surface inspection. Meanwhile, advances in detector resolution, valve technology, ejector-duct materials and design, will all help to optimise the ejection process. In the future, unwanted objects may be removed with rapier-like precision. Improvements to the operational stability of the sorting machine are likely to have a big impact, increasing the product throughput and ensuring that the machine optics need to be calibrated less frequently.

A consequence of the increased pace of technological advances will be a reduction in the working lifetime of sorting machines. New machines will have to be developed and manufactured under faster cycle times to keep pace with the market. Some components, particularly electronic chips, can quickly become obsolete. Similarly, the falling price of high technology is already allowing new competitors to enter the marketplace. Any optical sorting company that ignores

these factors can expect only reduced profit margins. In some ways, the real future challenge will be to provide integrated solutions that fulfil the demands of the food processing industry, at a price that can be justified.

## 6.19 Further reading

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## **Part II**

### **Colour control in food**



# 7

## The chemistry of food colour

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### 7.1 Introduction

If we ask the question 'why are foods coloured?', there are likely to be a number of answers. The plant and animal physiologists may respond as to the function of the colorant in the living tissue. The psychologist may respond in relation to the images different colours present to us. The aim of this chapter is, however, to consider how the chemist would respond to this question. This chapter aims to consider how the molecular structure of food colorants influences their colour, stability and reaction to other food components where appropriate.

Although it is common practice to refer to plant pigments and food dyes, the distinction between these in the food literature is not always clear. DeMann (1980) refers to pigments as 'a group of natural colorants found in animal and vegetable products'. In colour chemistry texts related to the textile industry, however, pigments are defined as being practically insoluble in the media in which they are applied (Herbst and Hunger, 1992) whereas dyes are soluble or partially soluble in the liquid in which they are applied. It is evident from this that the majority of our plant 'pigments' are water soluble, think of fruit juice drinks as a readily observable example, and as such (according to the textile colour chemists) should be referred to as dyes. The term colorant, however, is a collective term for all soluble or solubilized colouring agents, as well as insoluble pigments (DIN 6164, 1981). Thus in this chapter the term colorant will be used as a general term as any material (dye or pigment) which imparts colour to a food.



## 7.2 Classification of food colorants

Much of the detailed chemistry to understand food colorants comes from the textile and paint industry. Otterstätter (1999) and Christie (2001) state that colorants can be classified according to their chemical composition and method of application. The latter method of classification is detailed in The Colour Index (1988) and outlined by Otterstätter (1999), but is of limited use in our study of food colorants. In classification of food colorants two approaches are commonly taken, firstly, that based on the origin of the colorant and secondly, that based on chemical structure. In relation to origin this refers to whether a food colorant is natural, nature-identical or synthetic (Dalzell, 1997). Probably the best definition of a natural colorant is 'one which is synthesised, accumulated or excreted from a living cell' (Dalzell, 1997). Nature-identical colorants are those produced by a chemical synthesis to match the chemical structure of colorants found in nature. Synthetic colorants are those which are chemically synthesised and do not occur in nature. Proudlove (1994) considers that we should not use the term synthetic, but instead split food colorants into those naturally present in foods and those added to foods. This, however, also

**Table 7.1** Classification of natural colorants according to different authors

Main group <sup>a</sup>	Sub group or classification <sup>b</sup>
Isoprenoid derivatives <sup>1,2</sup>	Carotenoids <sup>1,2,3</sup> Xanthophylls <sup>1</sup>
Tetrapyrrole derivatives <sup>1,2</sup>	Chlorophylls <sup>1,2</sup> , Porphyrin <sup>3</sup> Heme pigments <sup>1,2</sup> Bilins <sup>2</sup>
Benzopyran derivatives <sup>1,2</sup>	Anthocyanins <sup>1,2</sup> , Flavones <sup>1</sup> Flavonoids <sup>2,3</sup> Tannins <sup>1</sup>
Artefacts <sup>2</sup>	Melanoidins <sup>2</sup> Caramels <sup>2,3</sup>
Others	Iso-allorazine <sup>3</sup> Phenalone <sup>3</sup> Betain <sup>3</sup> Anthraquinone <sup>3</sup> carbon <sup>5c</sup> Inorganic <sup>3c</sup>

<sup>a</sup> Main group taken from classification of Proudlove (1994) and DeMann (1980).

<sup>b</sup> Sub group as listed by either Proudlove (1994) or DeMann (1980) or classification given by Dalzell (1997).

<sup>1</sup> Classification given by different authors Proudlove (1994); <sup>2</sup> DeMann (1980) and <sup>3</sup> Dalzell (1997).

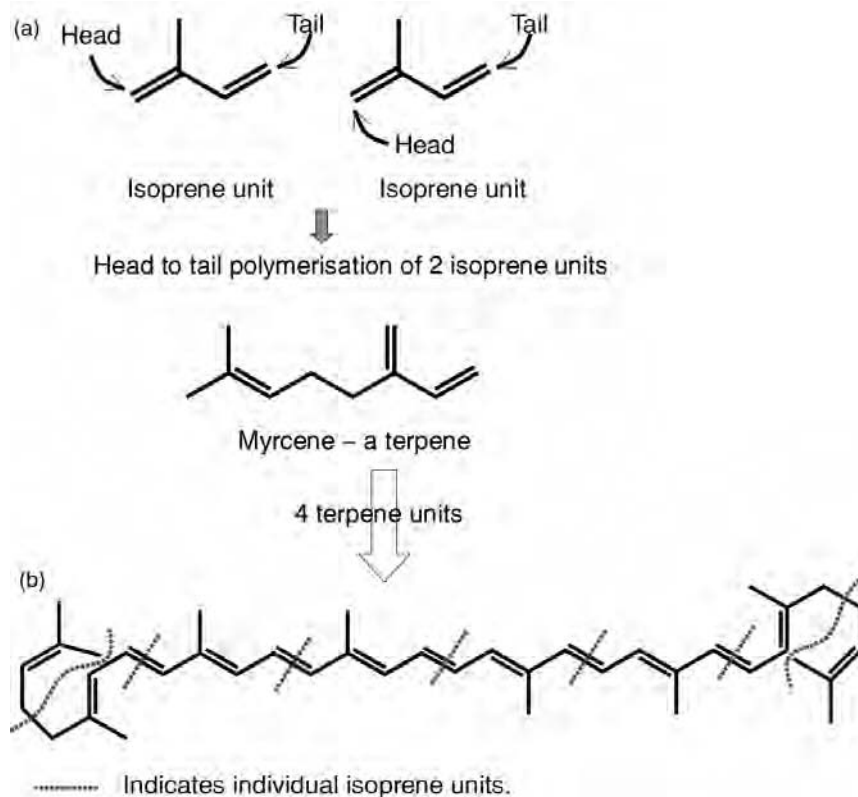
<sup>c</sup> – inorganic pigments and carbon not covered in this chapter.

has problems since 'those added to foods' may be natural, nature-identical or synthetic, although with increasing public pressure more natural or nature-identical colorants are being used. Proudlove (1994) does not clearly indicate what he means by a food, although it seems to apply to the raw food materials from plants or animals. If we consider it applies to processed foods we have another definition problem. Let us consider the colour of the crust of bread, this colour is produced by the Maillard reactions during the baking. Are the chemicals responsible for this 'natural' or 'synthetic' (certainly they were not added as colorants)? In many countries caramel is recognised as natural (Henry, 1996), but would not fit in with the definition of natural as given by Dalzell (1997).

The classification of food colorants on a chemical basis is also not always clear. Although Proudlove (1994) and DeMann (1980) agree on three chemical classes for natural food colorants that is, isoprenoid derivatives, tetrapyrrole derivatives and benzopyran derivatives, DeMann (1980) also includes a fourth group called 'artefacts' to include the melanoidins and caramels. Table 7.1 shows the classification based on chemical structure, the fourth group artefacts has been included as this is relevant to the discussion of chemical structure and light absorption. Table 7.1 also includes the names used by Dalzell (1997) in her classification.

### 7.3 Isoprenoid derivatives

In order to understand both the perceived colour in terms of its spectral properties and the chemical stability of the colorant we need to consider the chemical structure of the colorant. The isoprenoid derivatives (Fig. 7.1) are as the name suggests based on multiples of usually eight isoprene units ( $C_5H_8$ ). Their structure can be considered as a chain of two terpenoids, each terpenoid (a 10 carbon unit) being formed by two isoprene units being joined head to tail (see Fig. 7.1) to give a 20 carbon unit. These two 20 carbon units are condensed tail to tail to give a 40 carbon chain. Thus the carotenoids may be referred to as tetraterpenoids (see Fig. 7.1). The carotenoids may be classified using two systems. The first system classifies according to those that are hydrocarbons, the carotenes, and those which contain hydroxyl or keto groups, the xanthophylls (Fig. 7.2). The second system of classifying carotenoids is based on whether or not they contain cyclic elements in the form of a six membered ring at the end of the chain. Thus acyclic carotenoids such as lycopene do not contain any cyclic ring structure, monocyclic carotenoids contain one ring, e.g.,  $\gamma$ -carotene and bicyclic carotenoids contain 2 cyclic rings, e.g.,  $\alpha$ -carotene and  $\beta$ -carotene (see Fig. 7.2).  $\beta$ -carotene can be considered as a tail to tail bond of two vitamin A molecules. Figure 7.1b shows how the carotene chain is comprised of eight isoprene units. Further information on terpenes and the biosynthesis of carotenoids can be found in a number of organic and biochemistry texts (Brown, 1987; McMurry, 1988; Stryer, 1981). The structure of xanthophylls is



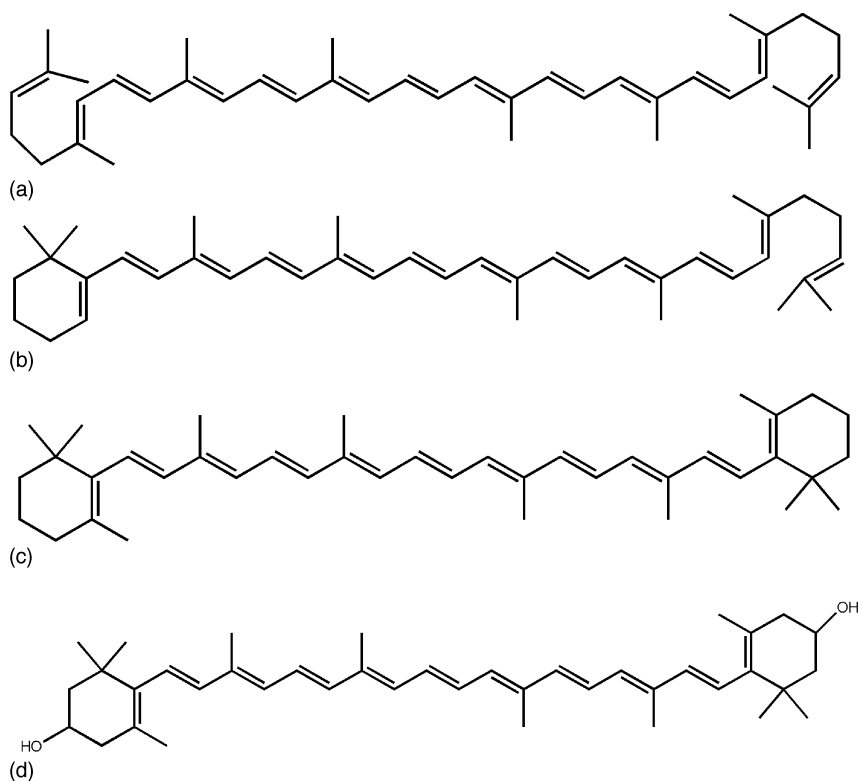
**Fig. 7.1** Isoprenoid derivatives (a) formation of a terpene from 2 isoprene units; (b) structure of lycopene – a tetraterpene showing the isoprene units.

essentially the same as the carotenoids but these contain hydroxyl groups, some examples are given by DeMann (1980). From the chemical structure and hydrocarbon nature of the chain (Fig. 7.2) it is evident that the carotenoids would tend to be fat soluble and not soluble in water. The hydroxyl groups of the xanthophylls tend to make them polar and more soluble in ethanol than the carotenes.

## 7.4 Benzopyran and tetrapyrrole derivatives

### 7.4.1 Benzopyran derivatives

The basic common structure of the anthocyanidins, flavins and tannins is the ring structure shown in Fig. 7.3a. The anthocyanidins consist basically of this structure with some hydroxyl groups through which are conjugated sugar molecules. The anthocyanidins refers to the molecule without the glycoside residue. The structure of flavonoids differs from anthocyanidins in that they



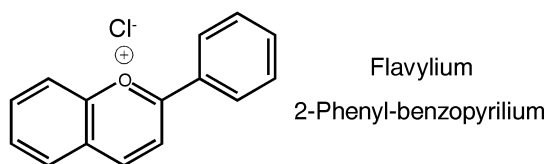
**Fig. 7.2** Classification and structure of carotenoids (a) lycopene – acyclic hydrocarbon; (b)  $\gamma$ -carotene – monocyclic hydrocarbon; (c)  $\beta$ -carotene – bicyclic hydrocarbon; (d) lutein – bicyclic xanthophyll.

contain a keto or hydroxyl group at position 4. The chemical structures of a range of flavonoids are given by DeMann (1980).

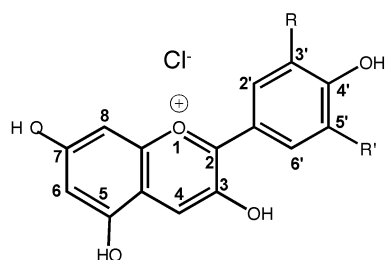
The tannins are used to describe a group of complex molecules found in tree bark and leaves of many plants. In the formation of black tea, through enzymic browning the polyphenol substrates are essentially flavonoids such as catechin (see Fig. 7.4a), which are converted to the corresponding  $\sigma$ -quinones. These quinones form dimers and further complex polymeric thearubigins. These thearubigins are the main components of black tea. The formula of the dimer theoflavin is given in Fig. 7.4b where the flavin structure can be clearly seen within the molecule. The brown of old wines is due to the formation of tannins through the polymerisation of the anthocyanins and other flavonoids.

#### 7.4.2 Tetrapyrrole derivatives

The common structural element in these molecules is a central metal atom surrounded by four linked pyrrole rings (hence tetrapyrrole) known as a

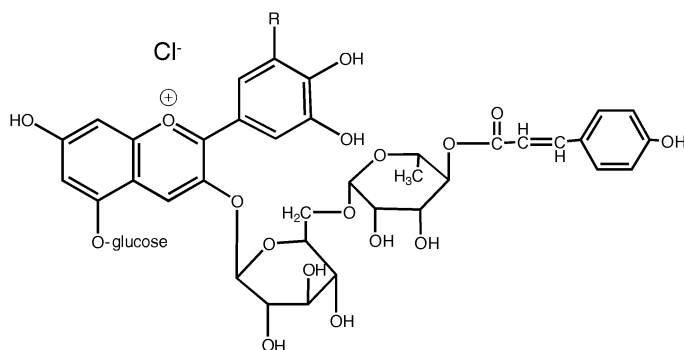


(a)



(b)

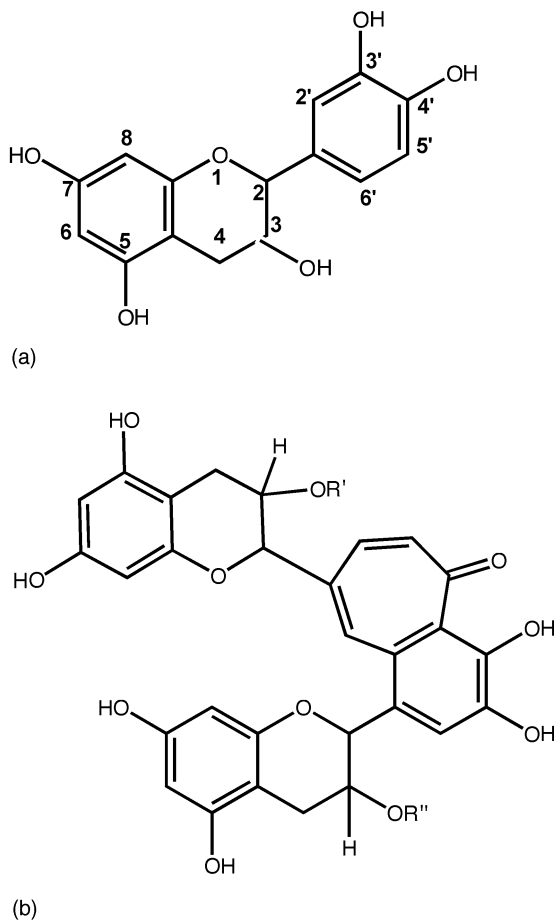
Name	R	R'
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH <sub>3</sub>	H
Delphinium	OH	OH
Petunidin	OCH <sub>3</sub>	OH
Malvidin	OCH <sub>3</sub>	OCH <sub>3</sub>



(c)

**Fig. 7.3** Benzopyran derivatives (a) flavan structure – basic structure of anthocyanidins (aglycosides of anthocyanins); (b) anthocyanidin structure; (c) structure of an anthocyanin showing conjugation with sugars.

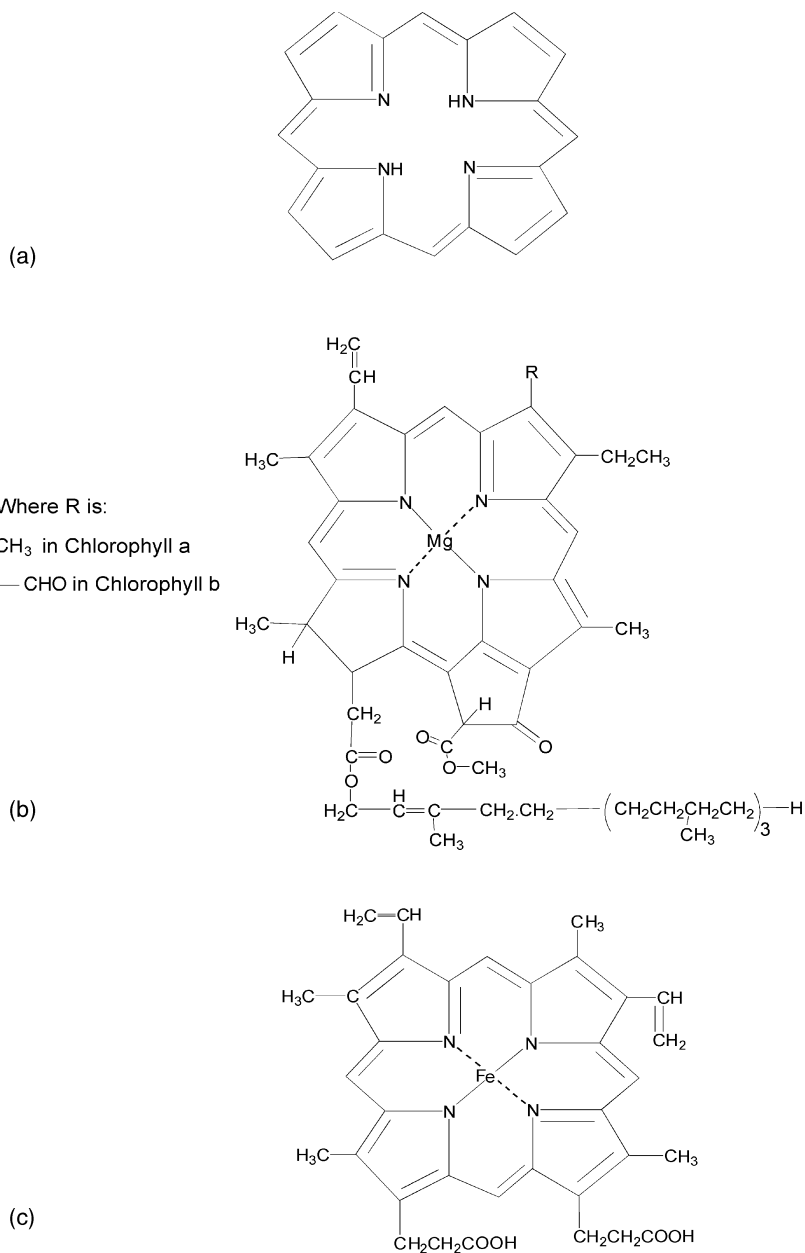
porphyrin ring. There are really two groups of compounds we need to consider: the chlorophylls where the central metal is magnesium and the hemichromes (hemoglobin and myoglobin), where the central metal is iron (see Fig. 7.5). The porphyrin ring is a planar structure and in the case of hemoglobin and myoglobin it forms the prosthetic group attached to the globular protein globin. The proximal histidine of the protein is coordinated to the iron (Fe(II) state) and other molecules such as oxygen, carbon monoxide can bind to the sixth coordination position. Thus the iron can be considered to be essentially in an octahedral coordination. The binding of different molecules to the sixth coordination position influences the spectra, and will be discussed later.



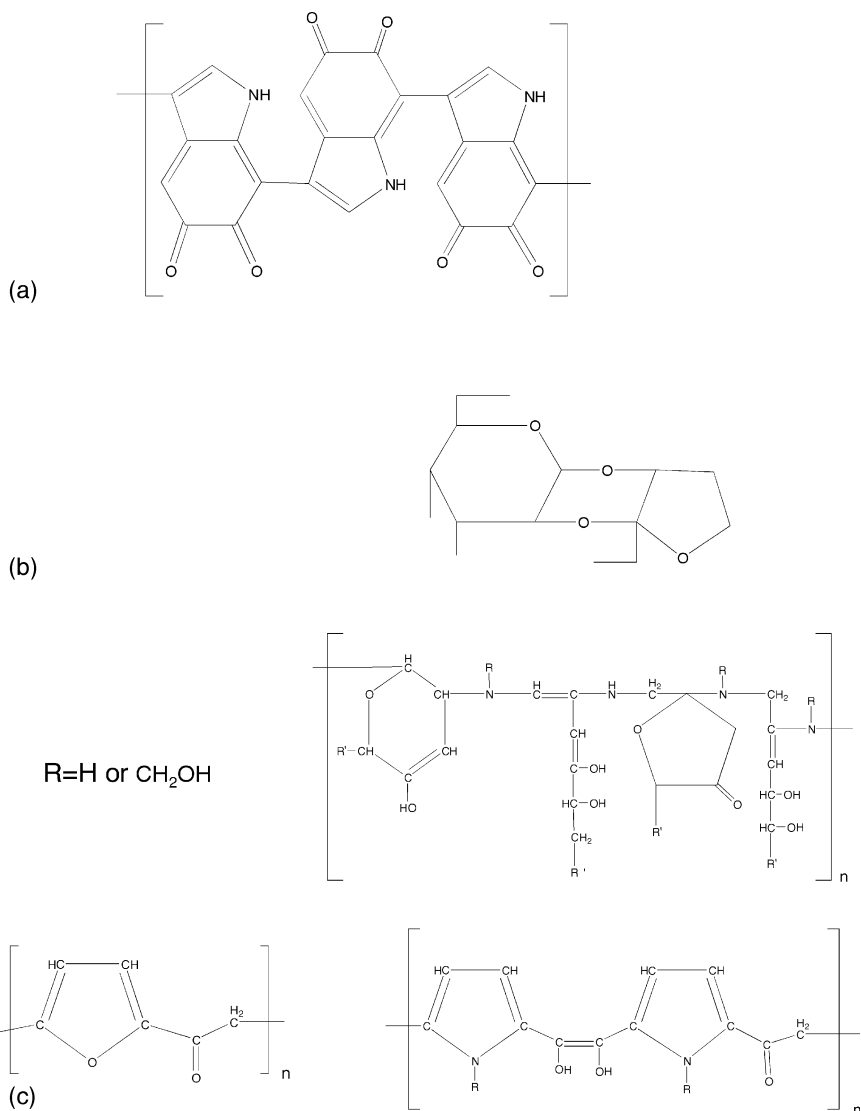
**Fig. 7.4** Tannins (a) catechin; (b) theaflavin.

## 7.5 Melanins, melanoidins and caramels

Melanins, melanoidins and caramels are grouped together since they are all complex polymeric molecules, with some similarity in structural units comprising the polymers (Fig. 7.6). In general their molecular structure has only been elucidated for a few cases. The distinction between the melanins and melanoidins and caramels is that melanins occur naturally and are responsible for many of the black, grey and brown colours found in plants and animals (Delgado-Vargas *et al.*, 2000). Melanoidins and caramels, however, are formed by non-enzymatic browning reactions, usually during the heat processing of foods. Melanoidins are produced as a result of Maillard reactions, essentially a reaction initiated between a reducing sugar and a primary or secondary amine (a protein amine group or an amino acid), while caramelisation reactions result



**Fig. 7.5** Tetrapyrrole derivatives (a) porphyrin free base; (b) chlorophyll; (c) Heme structure of myoglobin.



<sup>1</sup> From Tilley (1999). <sup>2</sup> Adapted from Wong (1989).

**Fig. 7.6** Melanins, melanoidins and caramels (a) proposed structure for eumalanin<sup>1</sup> – repeating unit of large polymer of brown melanins; (b) isosacchrosan proposed formula; (c) proposed structures for monomer structure of melanoidins.<sup>2</sup>

from the effect of heat on sugars. The chemical reactions and conditions for non-enzymatic browning reactions have been given by several authors (Coultate, 1988; Wong, 1989; DeMann, 1980). Although the initial stages of the various browning pathways of Maillard and caramelisation reactions differ, a scheme was proposed by Hodge (1953) which shows how these reactions (Maillard and



caramelisation) and indeed other non-enzymatic browning reactions relate with a number of common reaction pathways at later stages in the browning process.

Examples of the structure of melanins, melanoidins are given in Fig. 7.6. Many of the melanoidin structures have not been fully elucidated although intermediates have been identified particularly the low molecular weight volatiles giving rise to flavours produced during the reactions (Ames *et al.*, 1997, Farmer *et al.*, 1989). Also due to the complexity of non-enzymatic browning reactions in food materials during processing, where the amine group involved may be part of a peptide or protein, most of the research studies on the Maillard reaction have been done using simpler model systems. These model systems involve the reaction between a reducing sugar and an amino acid, e.g., xylose and lysine (Arnoldi *et al.*, 1997); glucose and lysine (Ames *et al.*, 1997); glucose and glycine (Yaylayan and Kaminsky, 1998). The polymeric products of Maillard reactions, i.e., the melanoidins are often characterised by structures containing 2 or 3 rings, where the rings may contain oxygen or nitrogen and are frequently linked by a  $-\text{CH}=\text{}$  group. Arnoldi *et al.*, (1997) identified a number of compounds from the reactions of xylose with lysine, which contained furan rings. These furan rings which are common in many of the coloured Maillard reaction products may derive from the electrophilic condensation on a nucleophilic carbon of furfural, the latter deriving from the decomposition of xylose (Arnoldi *et al.*, 1997). Ames *et al.* (1997) studying the reaction between glucose and lysine at 150°C identified a coloured compound with the empirical formula  $\text{C}_{19}\text{H}_{33}\text{N}_3$ . Yaylayan and Kaminsky (1998) gave a scheme showing a possible hypothetical polymerisation sequence during the Maillard reaction, they also proposed that monomeric units of the polymers contained furan rings. Although the structure of the compound formed in the first stage of caramelisation, isosaccharosan (see Fig. 7.6), was elucidated in 1924 by Pictet and Stricker, the structures of the compounds formed in the later stages, caramelan, caramelen and caramelin have not been elucidated and only their empirical formula has been elucidated.

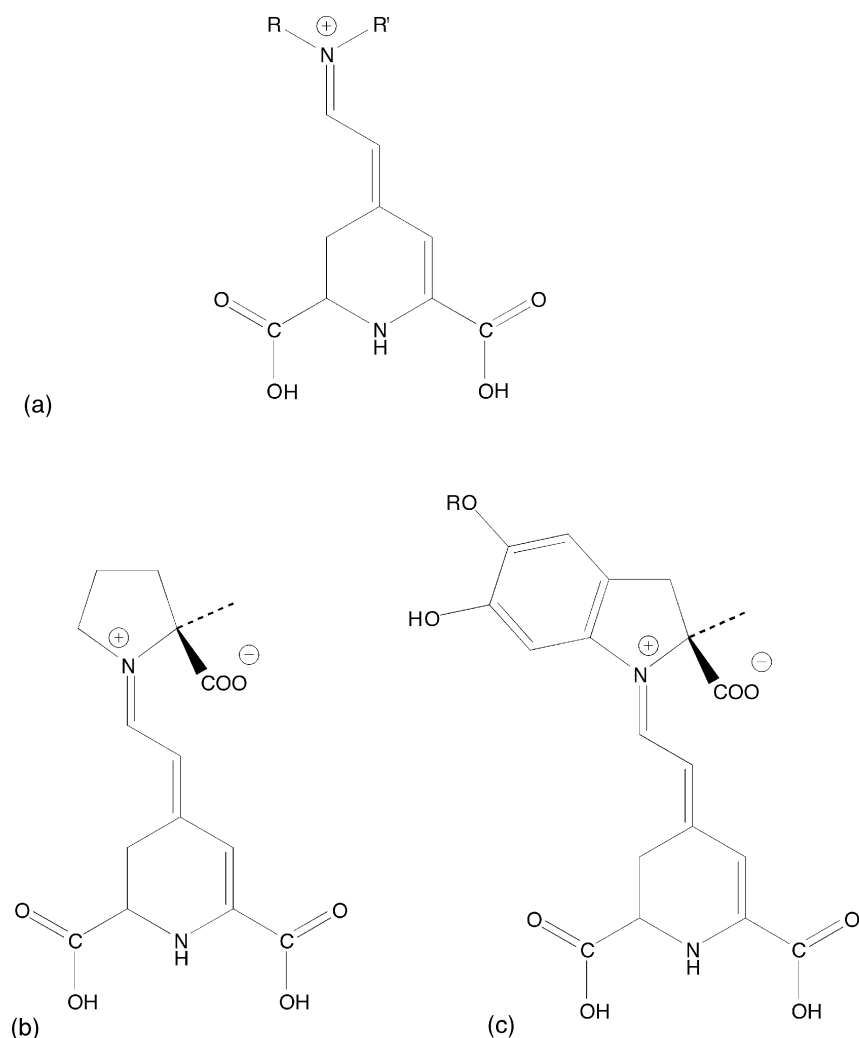
Since these non-enzymatic browning reactions can be used to produce both food colorants (melanoidins and caramel) and flavours we might consider classifying them as 'synthetic' hence the classification 'artefacts' given by DeMann (1980). It must be remembered, however, that many of our heat processed food products, e.g., crust of bread, brown crust on pastry products, toast, derive their appearance and flavour from non-enzymatic browning reactions. The colour and flavour of roasted coffee is due to non-enzymatic browning reactions and the colour and flavour of black tea is due to enzymatic browning reactions. In these two food products the melanoidins would be considered as 'naturally occurring'.

## 7.6 Other natural colorants

The classification given in Table 7.1 by DeMann (1980) and Proudlove (1994) does not embrace all possible natural colorants. One important category is the

betalains, the pigments extracted from beetroot (*Beta vulgaris*). These were previously described as nitrogenous anthocyanins as they contained nitrogen in their ring structures and also contained glycoside residues. These are now considered a separate group and can be further subdivided into the betacyanins (which are purplish red) and betaxanthins (which are yellow). The betaxanthins do not have an aromatic ring system attached to N-1 or sugar residues (Fig. 7.7).

The system of classification used by Dalzell (1997) includes two other groups of natural food colorants the phenalones (e.g., curcumin and turmeric) (Fig. 7.8); anthraquinone (Fig. 7.9) (cochineal carmine). Other naturally occurring coloured



**Fig. 7.7** Betalains (a) general structure; (b) betaxanthin structure; (c) betacyanin structure.

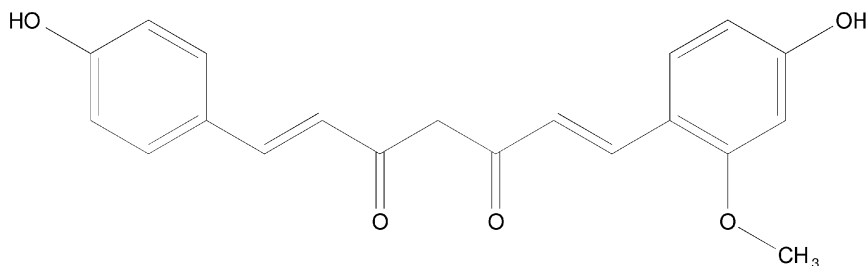


Fig. 7.8 Phenalene

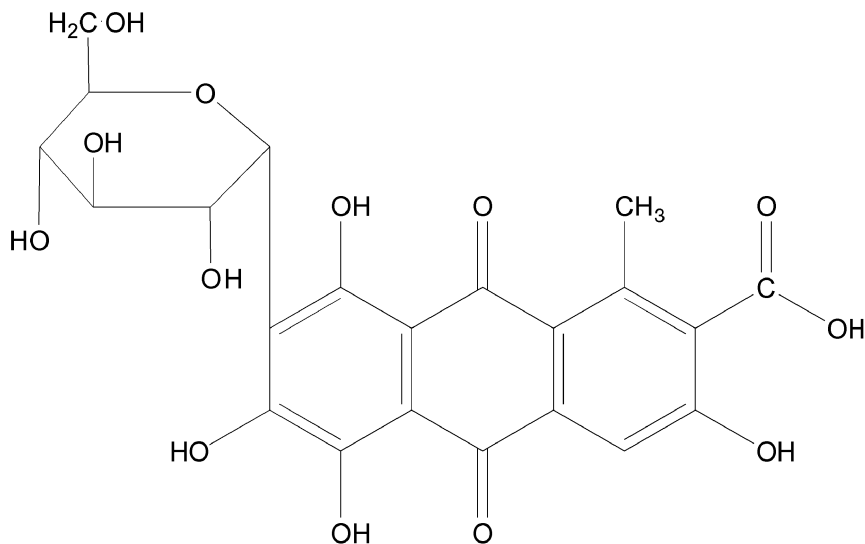


Fig. 7.9 An anthraquinone.

compounds such as riboflavin are also included, although these are less widely used as food colorants.

## 7.7 Chemical structure and light absorption

If we consider the chemical structure of the natural colorants we should be able to answer questions such as ‘why are they coloured?’, ‘why do they have different colours?’ and ‘how do they behave chemically in terms of stability, oxidation, pH?’. The first question why are they coloured could be answered by saying that these compounds absorb wavelengths of light selectively in the visible region. Such absorption is associated with energy changes within the molecule as described in the Einstein-Bohr equation (Eqn 7.1). This states that the energy difference ( $\Delta E$ ) between the ground state and a particular excited

state is directly proportional to the observed frequency ( $\nu$ ) and inversely proportional to the wavelength ( $\lambda$ ) of the absorbed light. To get an appreciation of the energy involved we can compare the colour changes in the correlated colour temperature, i.e., the temperature at which a Planckian radiator (black body) is observed as a particular colour. In the visible region the correlated temperatures range from around 2,000°K at the red end to 8,000 to 10,000°K at the blue end. The energy associated with the absorption of light can be calculated from the Einstein-Bohr equation if we assume that the visible spectrum ranges from 380 to 780nm and substitute these values in equation 7.2. The calculated energy change at 380nm is  $5.22 \times 10^{-19}\text{J}$  and at 780 nm is  $2.5 \times 10^{-19}\text{J}$ . We can then compare these energy changes with the energy associated with different molecular and electronic changes.

Einstein-Bohr equation:

$$E = h\nu \quad 7.1$$

where  $E$  = energy

$h$  = plancks constant ( $6.626 \times 10^{-34}\text{J s}$ )

$\nu$  = frequency in Hertz ( $\text{s}^{-1}$ )

or for wavelength ( $\lambda$ )

$$E = \frac{hc}{\lambda} \quad 7.2$$

where  $c$  = velocity of light ( $2.99 \times 10^8\text{m s}^{-1}$ )

$\lambda$  = wavelength (m)

for the lower limit of the visible region we have a wavelength of 380nm ( $\lambda = 380 \times 10^{-9}\text{m}$ ) therefore substituting we have:

energy change due to one photon

$$\Delta E = \frac{6.626 \times 10^{-34} \times 2.997 \times 10^8}{380 \times 10^{-8}} \quad 7.3$$

$$\Delta E = 5.22 \times 10^{-19}\text{J per photon}$$

for the upper limit of the visible region we have a wavelength of 780nm, thus substituting in Eqn 7.2 we have:

$$\Delta E = 2.5 \times 10^{-19}\text{J per photon absorbed}$$

We can obtain the energy per mole by using Avogadros number ( $N = 6.023 \times 10^{23}$ ).

Energy change per mole:

$$\Delta E_{mol} = \frac{Nhc}{\lambda} \quad 7.4$$

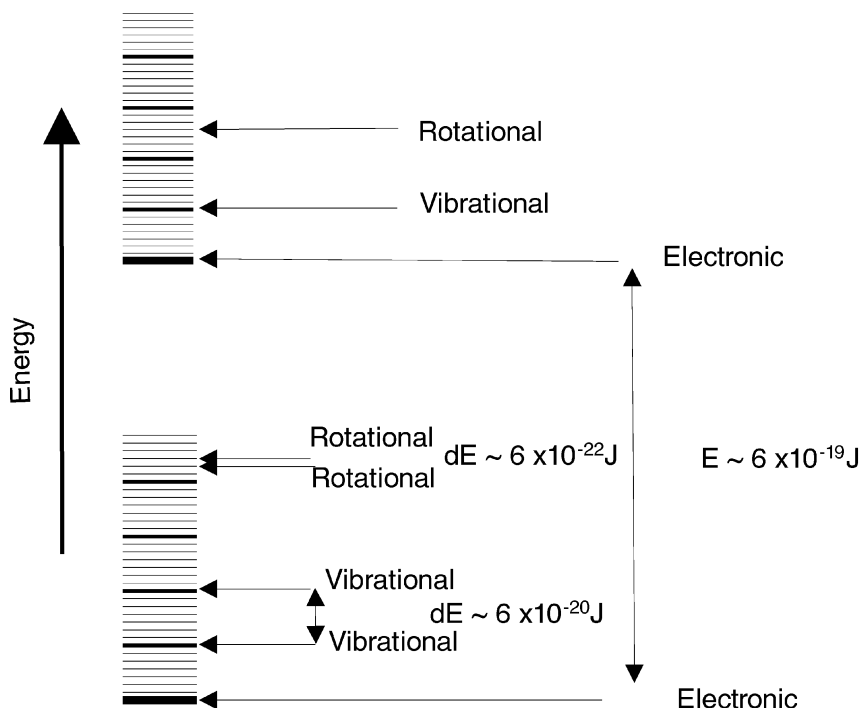
Thus the calculated values for the lower limit of the visible region (380nm) is:

$$\Delta E_{mol} = 314401 \text{ J mol}^{-1}$$

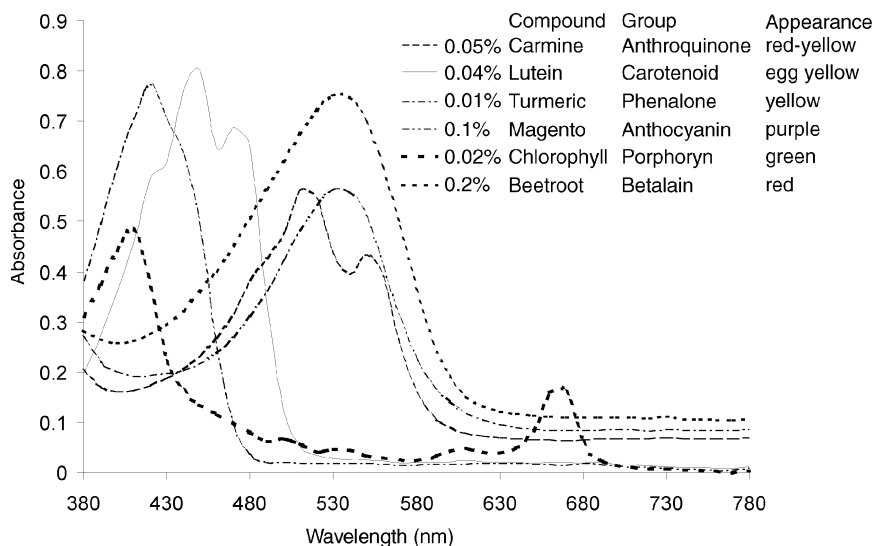
and for the upper region 780 nm

$$\Delta E_{mol} = 150575 \text{ J mol}^{-1}$$

Thus the calculated energy change is of the order of  $5 \times 10^{-19} \text{ J}$  (at the blue ultraviolet end) and  $2.5 \times 10^{-19} \text{ J}$  at the red end of the spectra. If we consider the structure of molecules then we have three different types of energy levels (1) electronic (2) vibrational and (3) rotational (Fig 7.10). The energy associated with the separation of these levels is  $6 \times 10^{-19} \text{ J}$  for electronic,  $6 \times 10^{-20} \text{ J}$  for vibrational and  $6 \times 10^{-22} \text{ J}$  for rotational (Fig. 7.10). The smaller energy transitions due to vibrational and rotational changes give rise to absorption in the infrared and microwave regions of the electromagnetic spectra. In molecules the overlap of the electron orbitals forming the bond can give rise to different energy transitions. Although often these chemical bonds are considered simply to be formed by the overlap of two atomic orbitals, it is more accurate to think of these overlapping orbitals to extend to some other atoms in the molecule to form molecular orbitals. This molecular orbital theory has been used to explain the colour of molecules. Before we try to rationalise the appearance of the food colorant structures (Figs. 7.1–7.9) we first need to consider the relationship



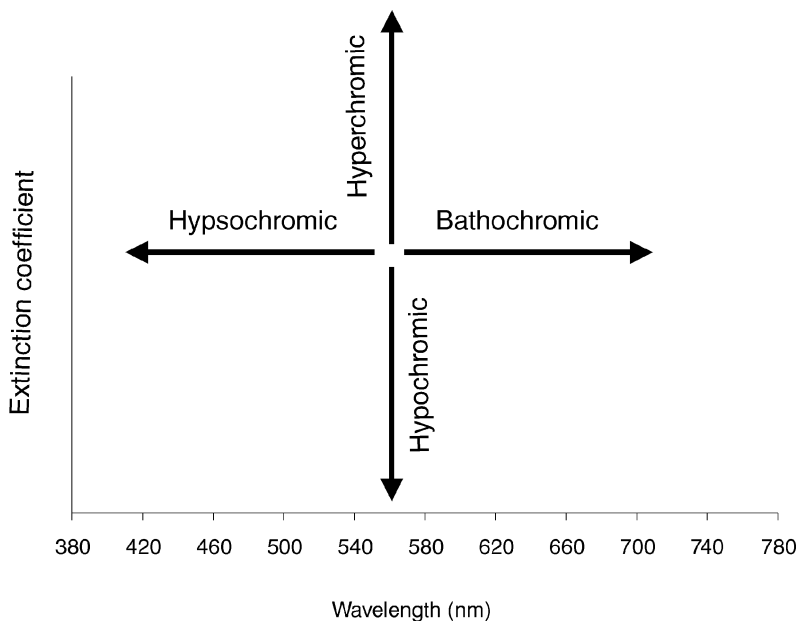
**Fig. 7.10** Schematic diagram of the electronic, vibrational and rotational energy level of a molecule.



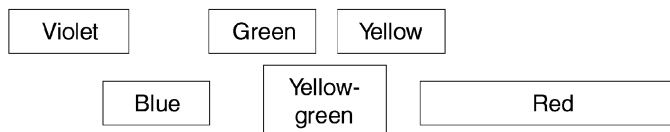
**Fig. 7.11** Spectra of some food colours.

between the wavelength absorbed and appearance. The spectra of some selected food colorants is shown in Fig. 7.11. Thus carotene which appears 'yellow/orange' has an absorption spectrum which shows that the maximum absorption wavelength ( $\lambda_{\text{max}}$ ) is around 497 nm. Fig. 7.12 gives the relationship colour absorbed, colour perceived and  $\lambda_{\text{max}}$ . The ability of the molecule to absorb light, i.e., what is referred to as its strength in the dyeing industry, is quantified by the extinction coefficient, the higher this is the greater the absorption, thus a smaller amount of colorant will be required to obtain the desired colour effect. A hyperchromic shift refers to an increase in extinction coefficient and hypochromic a decrease in extinction coefficient (Fig 7.12).

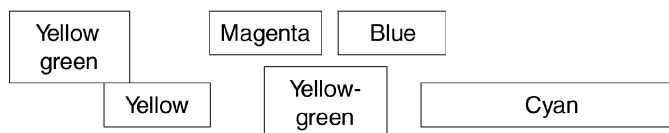
To apply the molecular orbital theory to food colorants (Figs 7.2 to 7.9), we should consider what common molecular structures the food colorants contain (i.e., why do they absorb in the visible region) and how do they differ, e.g., why is chlorophyll green and myoglobin red/purple yet they both are tetrapyrroles. The most common feature in all food colorants is the presence of a number of double bonds, some in hydrocarbon chains, others in ring structures (both 5 and 6 membered rings). The wavelength peak of the absorption band of a single carbon carbon double bond is around 160 nm, and increasing the number of double bonds from one as in compounds A, B and to two (Table 7.2) as in compounds D and F does not markedly change the wavelength peak ( $\lambda_{\text{max}}$ ). However, compound C which has the same number of double bonds as D has a higher wavelength. The difference between C and D can be explained by the fact that C is a conjugated diene and has a number of resonance structures (see Fig. 7.13). This can be explained by molecular orbital theory. The transition of



*Absorbed colour\**



*Observed colour\**

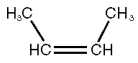
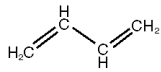
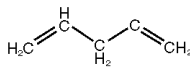
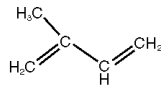
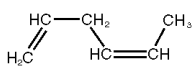
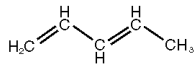
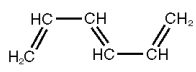
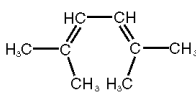
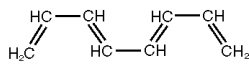
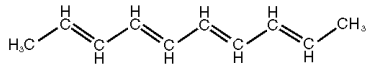


\* Names of colour absorbed and observed truncated from Tilley (1999). Note: descriptions of colour associated with wavelength range vary slightly according to author (see McLaren, 1986; Fieser and Fieser, 1964; Wong, 1989).

**Fig. 7.12** Relationship between wavelength of absorbed light and appearance of colorant.

electrons between  $\sigma$  and  $\pi$  orbitals is given in a number of texts (Jones, 1997; McMurry, 1988), however for coloured molecules it is the  $\pi$  molecular orbitals that are of most interest. If the electrons within an orbital contribute to the chemical bond within a molecule it is said to be a bonding orbital, this is referred to as the highest occupied molecular orbital (HOMO). The orbital with the next

**Table 7.2** Relationship between structure and maximum wavelength of absorption\*

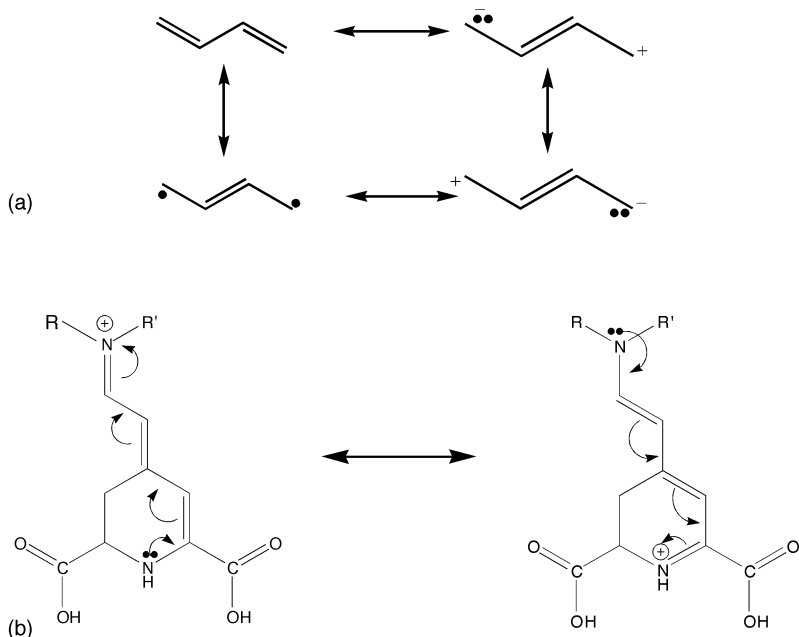
Carbon atoms	Double bonds	Structure	Name	$\lambda$ max* nm
A <sup>1</sup> 2	1	$\text{H}_2\text{C}=\text{CH}_2$	ethene	171
B 4	1		Cis 2-butene	170
C <sup>1</sup> 4	2		1,3-butadiene	217
D <sup>1</sup> 5	2		1,4-pentadiene	170
E <sup>1</sup> 5	2		2-methyl-1,3-butadiene	222
F <sup>2</sup> 6	2		1,4-hexadiene	170
G <sup>3</sup> 6	2		1,3-pentadiene	223
H <sup>1</sup> 6	3		<i>trans</i> -1,3,5-hexatriene	268
I <sup>1</sup> 8	2		2,5-dimethyl-2,4-hexadiene	241
K <sup>1</sup> 8	4		<i>trans,trans</i> -1,3,5,7-octatetraene	330
J <sup>4</sup> 10	4			296

Source:

<sup>1</sup> Vollhardt (1987); <sup>2</sup> McLaren (1986); <sup>3</sup> McMurray (1988); <sup>4</sup> Tilley (1999).\*  $\lambda$ max is the wavelength of the strongest peak, there may be more than one peak in the spectra.

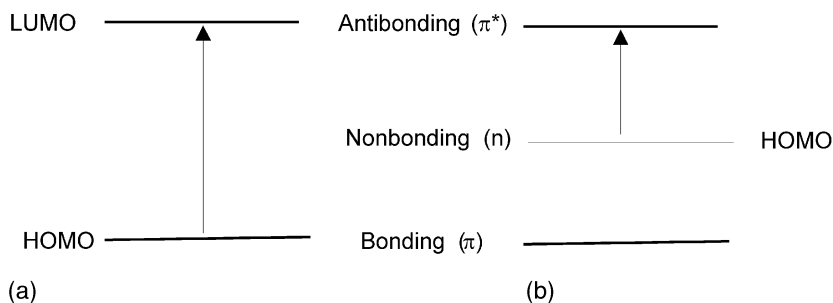
highest energy ( $\pi^*$ ) is the orbital which does not contain any electrons, this is referred to as the lowest unoccupied molecular orbital (LUMO) (Fig. 7.14). The transition of electrons between  $\pi$  and  $\pi^*$  orbitals are the ones usually involved in the visible region. Between the bonding orbital ( $\pi$ ) and antibonding orbital ( $\pi^*$ ) there is an intermediate energy level of a non-bonding orbital (n). These contain electrons which do not contribute to the bonding in the molecule, and may consist of a lone pair of electrons, for example associated with a nitrogen atom





**Fig. 7.13** Resonance structures in some food colorants (a) conjugated double bonds, e.g. carotenoids; (b) due to lone pair electrons from nitrogen, e.g., betainins.

and in this case would be the highest occupied molecular orbital (HOMO). Transitions for  $n$  (HOMO) to  $\pi^*$  (LUMO) are important in molecules containing ketone groups ( $>C=O$ ). As a general rule in conjugated dienes, without strong electron withdrawing or electron donating groups, the energy of the  $\pi$  to  $\pi^*$  transition decreases as the number of resonance structures increases and the number of conjugated double bonds increases. A shift in wavelength absorption



Note: The electronic transition depends on the molecule and the orbitals occupied in the ground state, for detailed diagrams of bonding and electronic transitions for methanal and 1,3-butadiene see McDonald (1997) and Wong (1989).

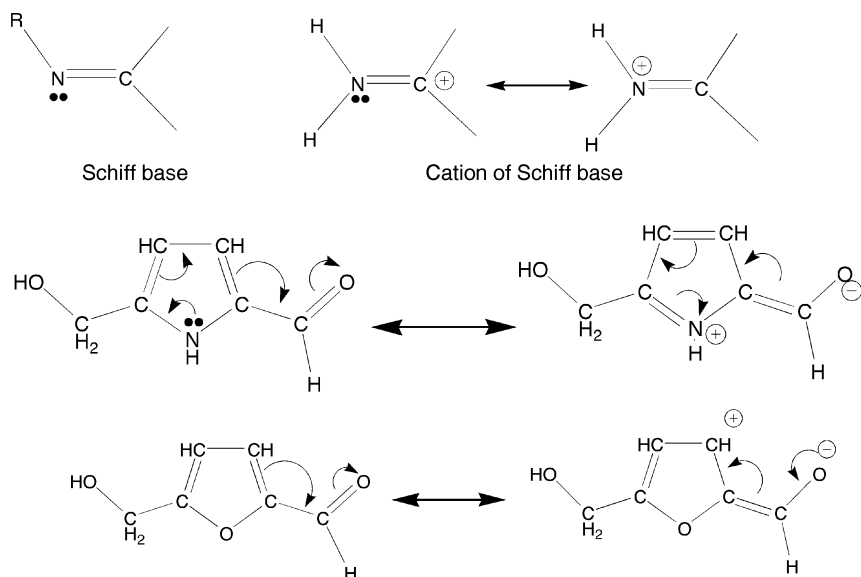
**Fig. 7.14** Electronic transactions in molecules (a)  $\pi$  to  $\pi^*$  transition; (b)  $n$  to  $\pi^*$  transition.

towards a higher wavelength is termed a bathochromic shift and to lower wavelength a hypsochromic shift. Increases in intensity of absorption (i.e., extinction coefficient) are hyperchromic and decreases in extinction coefficient hypochromic. These relationships are summarised in Fig. 7.12. It should be noted that many of the food colorants do not have just one single absorption peak and thus the relationship between  $\lambda_{\text{max}}$  absorbed and the colour perceived is a generalisation. A striking example of this is a comparison between chlorophyll which appears green, and myoglobin which appears red/purple, yet both have high absorption (high extinction coefficient) in the 420 to 440 nm region (this will be discussed later).

## 7.8 Molecular orbital theory and food colorants

It is evident then that we can explain the colour changes in the carotenoids fairly easily by applying the molecular orbital theory. Wong (1989) shows how both the energy and  $\lambda_{\text{max}}$  and extinction coefficient ( $\epsilon$ ) can be calculated for conjugated dienes using Fieser-Kuhn rules. The incorporation of groups into the molecule which increase resonance will reduce  $\Delta E$  and hence increase  $\lambda_{\text{max}}$  and result in a bathochromic (red shift) shift. The term 'red shift' often used to describe a bathochromic shift can be misleading as it refers to the wavelength absorbed not perceived. These groups also tend to increase extinction coefficient ( $\epsilon$ ) and are termed auxochromes (McLaren, 1986). Such groups are commonly seen as constituents of synthetic colorants, and indeed some of the earlier colour chemistry theories suggested that absorption in the visible region was due entirely to the presence of chromophores and auxochromes (Witt, 1876). Witt considered the chromophores to be the group of atoms within the molecule responsible for the colour, and considered the auxochromes groups which enhanced the colour of the molecule (Christie 2001). Chromophores are generally electron withdrawing groups (e.g.,  $-\text{CH}=\text{}$ ,  $\text{NO}_2$ ,  $\text{C}=\text{O}$ ,  $-\text{N}=\text{N}-$ ) and auxochromes electron donating groups (e.g., hydroxyl groups  $\text{OH}$ , amino groups  $\text{NR}_2$ ) (McLaren, 1986). Groups with lone pairs of electrons such as,  $-\text{OR}$  (where  $\text{R} = \text{CH}_3$ ,  $\text{C}_2\text{H}_5$ ),  $-\text{X}(\text{Br}, \text{Cl})$ ,  $-\text{NH}_2$  and electron withdrawing groups such as  $-\text{COR}$ ,  $-\text{CN}$ ,  $-\text{COOH}$ ,  $-\text{CHO}$ ,  $-\text{NO}_2$ ,  $-\text{COOH}$ ,  $-\text{SO}_3\text{H}$  also increase resonance through  $\pi$  conjugation (Wong 1989). Anthocyanins have both conjugated and substituent groups and the effect of pH changes and subsequent charge status of the oxygen changes the number of resonance structures and hence results in changes in absorption spectra, and colour may be lost at high pH values. The Betalains contain nitrogen (lone pair electrons) both as part of a heterocyclic ring and also a substituent group and show resonance structures (see Fig. 7.13).

For the melanoidins and caramels, given that these are complex polymeric molecules and their structure has only been elucidated in a few cases (Arnoldi *et al.*, 1997), it is difficult to show possible resonance structures. An examination of the proposed pathways for non-enzymatic browning give intermediates in which resonance structures are important. This includes keto-enol trans-



**Fig. 7.15** Possible resonance structures for Maillard reaction intermediates.

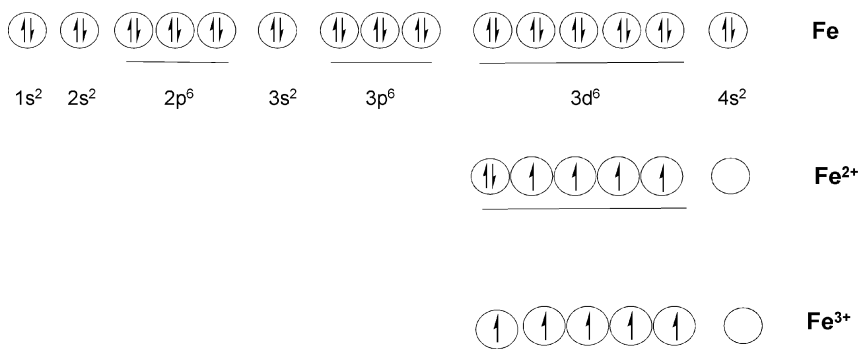
formations and other rearrangements (Wong, 1989; Coultate, 1988). The structure of eumalin (Fig. 7.6) clearly shows both conjugation and substituted groups which would be electron withdrawing. Examples of resonance structures for some of the intermediates in the Maillard browning reaction are given in Fig. 7.15. Examination of the proposed structures for melanoidins given by DeMann (1980) Fig. 7.6c and Yaylayan and Kaminsky (1998) Fig. 7.15, shows that these structures contain both conjugated systems and electron donating and electron withdrawing groups that would act as chromophores. Melanoidins have a strong absorption in the UV region as well as the visible region. Monzocco *et al.* (2001) summarises a number of papers relating antioxidant activity to colour and Maillard browning and shows that most studies have used a wavelength of around 450 nm (from 420 to 480) to quantify the brown colour formed. The coloured Maillard compounds studied by Arnoldi *et al.* (1997) had maximum absorption peaks ( $\lambda_{\text{max}}$ ) in the UV at 245 and 330 nm and in the visible region at 410 nm and the structures proposed showed conjugated systems. From the colour chemistry and spectral properties the high absorption of the melanoidins at 450 nm could be compared with the high absorption of myoglobin in the soret region (around 420 nm), which also appears red/brown.

The effect of spectral changes, and evidence of the consequent increase in conjugation and resonance structures during the formation of melanoidins is shown in the data presented by MacDougall and Granor (1998). They showed that as the sugar-amino acid mixtures were heated the maximum absorption peak ( $\lambda_{\text{max}}$ ) increased from around 200 nm at the start (due to the amino acid), after 0.25h the  $\lambda_{\text{max}}$  was around 400 nm and around 600 nm after 1h. There was a

marked increase in extinction coefficient such that after 1h incubation there was virtually no light transmitted in the UV/visible region between from 200 nm to 650 nm. MacDougall and Granor (1998) also found the absorption spectra of melanoidins from xylose-lysine and glucose-lysine mixtures were similar over the spectral range measured. This raised the question of whether it is the amino acid or the sugar which has the controlling role in the character of the colorant formed. Their data would indicate that maybe the amino acid has the controlling role. Further work however, is needed both on the elucidation of the structural formula of the melanoidins and also how the absorption spectra relates to conditions of reaction, e.g. type of sugar, amino acid, temperature, pH without other studies showing full spectra of the melanoidins formed.

Applying molecular orbital theory to the tetrapyrrole group of food colorants (chlorophyll and myoglobin), it can be clearly seen that the porphyrin ring has a conjugated structure. Free base porphyrins (Fig. 7.5) show strong absorption in the 550 to 600nm region and a strong band at around 400 nm. The coordination of the metal in the centre of this planar porphyrin ring and the electronic orbital configuration of the metal needs to be considered. The coordination of a metal introduces additional resonance structures. In chlorophyll the central metal is an alkaline earth Mg (electronic configuration  $1s^2, 2s^2, 2p^6 3s^2$ ) whereas in myoglobin it is a transition metal Fe (electronic configuration  $1s^2, 2s^2, 2p^6 3s^2 3p^6 3d^6 4s^2$ ). To study the electronic changes fully it is necessary to consider the principles of coordination chemistry and the role of the d orbitals in forming complexes (Cotton and Wilkinson, 1980). Figure 7.16 shows the electronic structure of Fe,  $Fe^{2+}$  and  $Fe^{3+}$ . Details of the energy of the different orbitals of Fe and background to coordination chemistry is given by Wong (1989). We can consider that in both chlorophyll and myoglobin we have a metal ion at the centre of an electron donating structure (the porphyrin ring). As in the conjugated dienes we considered  $\pi$  to  $\pi^*$  transitions within the tetrapyrrole molecule, we now need to consider electronic transitions between the porphyrin ring and the metal and electronic transitions within the metal, particularly the transitions within d orbitals of Fe in myoglobin. In myoglobin the iron in the +2 state is coordinated in an octahedral manner to the four nitrogens of the porphyrin ring and covalently linked to the proximal histidine of the globin protein.

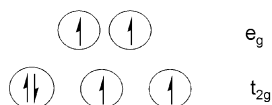
The sixth coordination position is closely associated with a distal histidine but it is this position to which other ligands such as  $O_2$ , CO and NO can attach. The 5 d orbitals of the iron can be grouped into 3 orbitals of lower energy (dxy, dyx, dxz) and 2 orbitals of higher energy ( $dz^2, dx^2-y^2$ ). In a coordination complex the energy difference between the two sets of d orbitals depends on the ligand complexed to the metal and this can also affect the geometry of the coordination complex (Wong 1989). For myoglobin in the deoxygenated state (deoxymyoglobin) there is one d orbital containing paired electrons with the remaining 4 orbitals containing single electron each. The binding of oxygen at the sixth coordinate position increases the energy difference between the d orbitals, since oxygen is a strong ligand. Thus in this case the 3 lower energy d



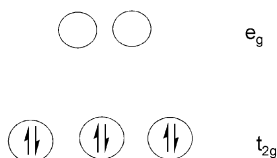
## Electronic configurations of the iron in Heme complex

(1) Deoxymyoglobin Fe<sup>2+</sup>

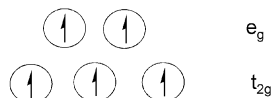
ligand H<sub>2</sub>O is not strong ligand energy difference between e<sub>g</sub> and t<sub>2g</sub> d orbitals is small, the 6 d electrons occupy all orbitals  
Paramagnetic due to unpaired electrons

(2) Oxymyoglobin Fe<sup>2+</sup>

ligand O<sub>2</sub> is a strong ligand, energy difference between e<sub>g</sub> and t<sub>2g</sub> d orbitals is large, the 6 d electrons occupy the three lower energy orbitals  
Diamagnetic due to paired electrons

(2) Metmyoglobin Fe<sup>3+</sup>

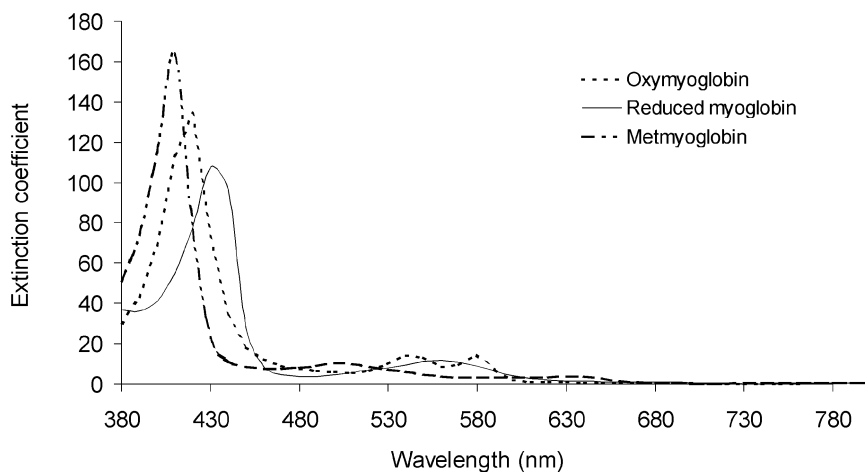
ligand H<sub>2</sub>O is not a strong ligand, energy difference between e<sub>g</sub> and t<sub>2g</sub> d orbitals is small, the 5 d electrons occupy all five d orbitals  
Paramagnetic due to unpaired electrons



**Fig. 7.16** Electronic configuration of iron and Heme complexes.

orbitals are filled with paired electrons. The resultant electronic orbitals from deoxymyoglobin, oxymyoglobin and ferrymyoglobin are shown in Fig. 7.16. Diagrams showing the energy levels of the orbitals and the bonds formed are given by Wong (1989).

In terms of energy transitions and absorption peaks the most marked difference is the splitting of the peak at 557 nm of deoxymyoglobin into two peaks at 544 and 582 nm in oxymyoglobin (Millar *et al.*, 1996). Oxidation of the iron (to Fe<sup>3+</sup>) results in a change in the deoxymyoglobin peak at 555 to a smaller one at 505 nm, which would indicate the electronic transitions required greater energy and also a small peak at 634 nm (see Fig. 7.17). Changes in spectra due to other ligands (e.g., H<sub>2</sub>S, NO<sub>2</sub>) have been studied by Millar (1994). The



Note: All three forms of myoglobin have a high absorbance in the 420–440 nm region (Soret peaks).

**Fig. 7.17** Spectra of myoglobin with different ligands and oxidation state of central iron.

protein globin stabilises the steric and electronic configuration of the molecule through hydrophobic interactions and hydrogen bonding. Changes in protein conformation also influence the shape of the absorption spectra (Millar, 1994). Although both the ligand, oxidation state and globin denaturation affect the soret region (400–440 nm), the effect on the spectra from 500 nm to 780 nm is often more marked (Millar, 1994).

In chlorophyll the magnesium has four coordination positions to the porphyrin ring however this is not a planar structure due to the presence of an alicyclic ring with carbonyl constituents. The magnesium in the chlorophyll structure can accept another electron donor ligand in one of the axial positions. Bifunctional nucleophilic ligands such as  $\text{H}_2\text{O}$  and  $\text{CH}_3\text{O}^+$  can act both as electron donor and also bridge to other chlorophyll molecules. The substitution of a methyl group ( $-\text{CH}_3$ ) in chlorophyll a to a formyl group in chlorophyll b ( $-\text{CHO}$ ) results in an increase in wavelength (decreases in  $\Delta E$ ) of the soret peak from 430 to 453 nm and also an increase in extinction coefficient (hypochromic shift). This bathochromic shift might be explained from an increase in resonance structures due to the formyl group. The second peak at 662 nm in chlorophyll a, has moved in the opposite direction to 642 nm in chlorophyll b with a decrease in extinction coefficient.

## 7.9 Chemical stability of food colorants

The common molecular features which give rise to absorption in the visible region, i.e., a conjugated double bond system, electron withdrawing and donating constituents and in the case of tetrapyrroles reactivity and oxidation

state of the central metal, will be crucial to the reactivity and stability of food colorants. The main practical aspects we need to consider are in relation to food processing are oxidation, pH, thermal degradation and additives.

### 7.9.1 Reactivity of conjugated dienes

In the previous section it was shown that one of the common features of food colorants was a conjugated diene structure. Thus in considering food colorant stability it is worth considering the typical reactions of a conjugated double bond system. The conjugated dienes show chemical reactions similar to alkenes, but with some important differences. The resonance structure of the conjugation makes them more stable and the heat of hydrogenation is less than a comparable non-conjugated system (Morrison and Boyd, 1967). We must, however balance against this the number of double bonds in the conjugated system, for example in the carotenoids (Fig. 7.2), which would give more opportunity for reaction. In the conjugated system the electrophilic addition (i.e., by HBr) results in both 1,2 and 1,4 addition. The mechanism of this can be explained though a resonance stabilised allyl cation (Jones, 1997).

### 7.9.2 Oxidation

It is well established that unsaturated fatty acids undergo oxidation, via a radical reaction mechanism. Carotenoids undergo similar reactions and indeed do this so readily they can act as antioxidants in food materials. This antioxidant ability of carotenoids derives from their ability to form a resonance stabilised free radical. In certain controlled conditions chemical oxidation of carotenoids can give rise to epoxide formation and isomerisation of this to a furanoxide (Wong, 1989). The epoxide formation has been shown to occur in canned fruit juice and can give rise to considerable loss of colour. This loss of colour can be accounted for by the reduced resonance stabilisation of the product, there is basically a loss of two conjugated double bonds, one in the 6 membered ring and alternate to this (Wong, 1989).

The benzopyran derivatives are not as easily oxidised as the carotenoids. In the heme tetrapyrrole derivatives the central iron atom may be oxidised relatively easily to form metmyoglobin. This change from  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  is observed easily when beef is exposed to the atmosphere for 2–3 days at refrigeration temperatures and faster at higher temperatures (MacDougall and Powell, 1997). The nature of the orbital changes for this have been given in Fig. 7.16.

In chlorophyll where  $\text{Mg}^{2+}$  is the central atom it is oxidation of the porphyrin ring which occurs. Under mild oxidation conditions (e.g.  $\text{KMnO}_4$  and acetone) the vinyl group of chlorophyll a and b is oxidised to carboxylic acid substituents. Under strong oxidising conditions such as chromic acid a mixture of pyrroles results (Wong, 1989).

### 7.9.3 Effect of pH

Since carotenoids are not soluble in water we do not need to consider pH effects for this group of food colorants. Of the other groups of food colorants we would expect pH effects to be greatest where the molecule contains ionisable groups. The most obvious group here is the anthocyanins which have the flavylum cation structure with a charged oxygen atom.

In low pH ( $\text{pH} \approx 1$ ) the colour of anthocyanins is red ( $\text{AH}^+$ , Fig. 7.18) as the pH is increased the anthocyanin may undergo two possible pathways (1) deprotonation to result in a blue quinoidal compound (A) or (2) hydration to

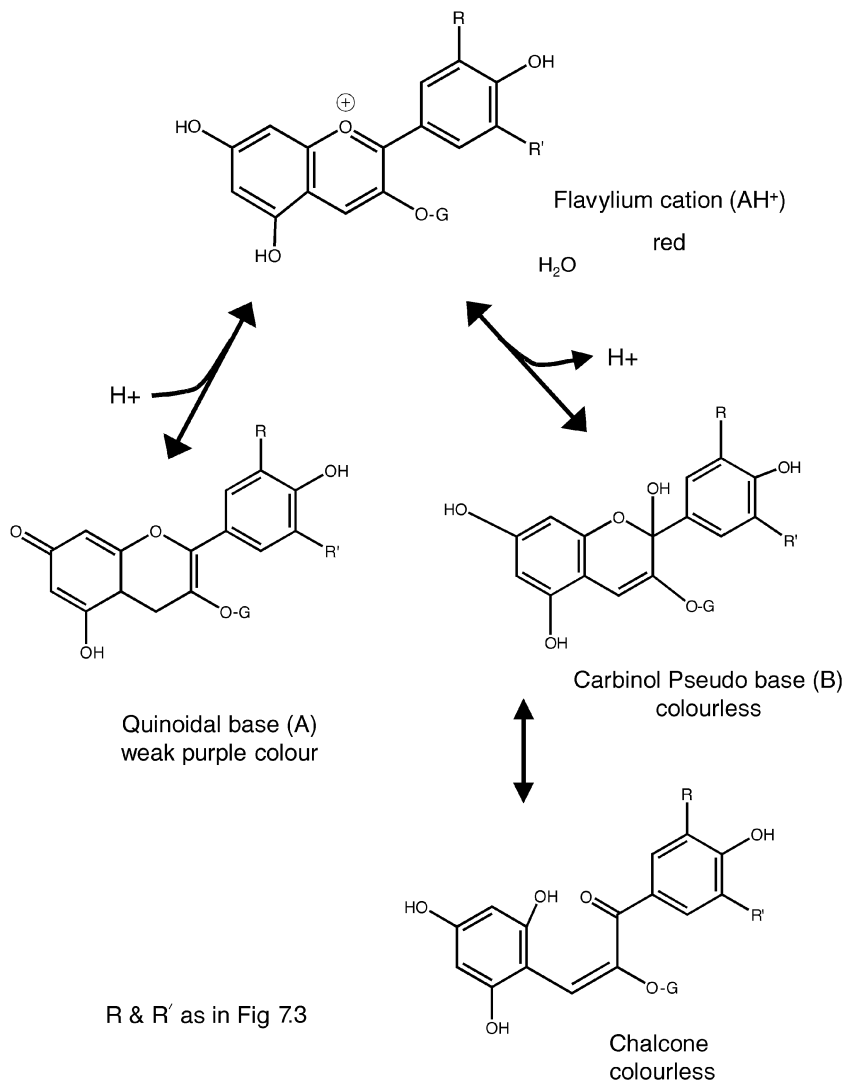


Fig. 7.18 Effect of pH on anthocyanin structure and colour.



result in a chalcone (C). In the case of A B and C compared to (AH<sup>+</sup>), there would appear to be a loss in resonance structure and less  $\pi$ - $\pi^*$  transition to account for the move to the blue end of the spectra. The absorption spectra show that as pH increases there is a marked decrease in the absorption peak at 515 nm with very little change in peak position, thus pH results mainly in a hypochromic shift. Several theories have been proposed to explain changes in extinction coefficient, probably the best approach is that of Poriser-Pole-Parr (PPP) (see Christie, 2001 for discussion). Molecules with high intensity (high extinction coefficient) are those in which the  $\pi$ - $\pi^*$  transitions are highly probable. The increase in pH has made these transitions less probable, and in trying to explain how the p orbitals extend over the conjugated system, we must think also of the 3-dimensional structure of the molecule. For example the change in phenolphthalein indicator from colourless in acid solution to red in alkaline solution is associated with both an increase in resonance structure and the formation of a more planar structure. The more planar structure allows the pi electrons to spread more evenly over the molecule.

The colour of myoglobin solutions are influenced by pH. In this case it is the pH effect on the globin protein that needs to be considered. In cured meat (nitrosylmyoglobin) denaturation of the protein results in conformational changes which allow a second NO ligand to attach to the iron forming a dinitroferrohemochrome (Wong, 1989).

## 7.10 Thermal stability

In relation to food products thermal stability might be considered in different broad temperature ranges: refrigeration to ambient for various storage conditions; 60 to 120°C to cover boiling, pasteurisation, sterilisation and 180 to 220°C for oven cooking, grilling and frying. It must be remembered that '*in vitro*' studies on the rate of reaction of individual compounds may not be a true reflection of their behaviour in complex food systems. Although increasing the temperature increases the rate of reaction as given by the Arrhenius equation (Eqn 7.5), it may also change the course of the reaction. Thus in the example of electrophilic substitution of conjugated dienes, the balance between the 1,2 substitution and 1,4 substituted product changes. At lower temperatures there is more of the 1,2 substitution than 1,4 substitution (Jones, 1997). The effect of heat carotenoids to 190°C (e.g., frying, oven cooking) results in a number of degradation products (Fig. 7.19). The formation of these cyclic products involves a four membered ring structure (Schweiter *et al.*, 1969).

Arrhenius equation:

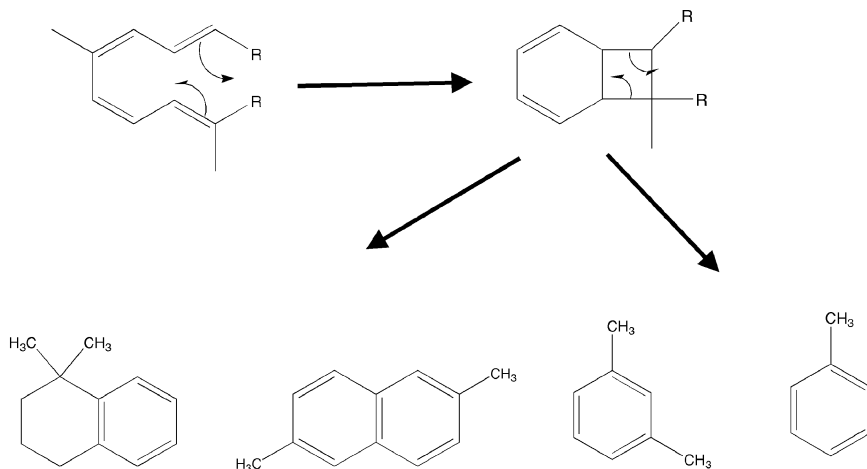
$$k = Ae^{-Ea/RT} \quad 7.5$$

$$\ln k = \ln A - Ea/RT \quad 7.6$$

where  $k$  = rate coefficient ( $\text{min}^{-1}$ )  
 $E_a$  = Activation energy ( $\text{J mol}^{-1}$ )  
 $A$  = constant ( $\text{min}^{-1}$ )  
 $R$  = Gas constant ( $\text{J mol}^{-1} \text{K}^{-1}$ )

Dyrby *et al.*, (2001) studied the effect of heat ( $20^\circ\text{C}$  to  $80^\circ\text{C}$ ) on anthocyanin extracts from a range of plant materials (red cabbage, blackcurrant, grape skins, elderberry) and in different media (buffer, carbonated soft drink). Over the 6h period of their study they found that the data could be fitted to first-order rate law as found by others (Cemeroglu, 1994; Sapers *et al.*, 1981). In both pH3 buffer and carbonated drinks the order of stability was red cabbage extract > blackcurrant > grape skin > elderberry and, in general, the rate of degradation in the carbonated system was twice as high as the pH3 buffer system. This shows the importance of studying changes in a relevant food system. In this case the decrease in thermal stability in the carbonated system could be attributed to the sugar and ascorbic acid in the carbonated drink system (Markakis, 1982). For practical use as a food colorant the excellent stability of the red cabbage extract (Dyrby *et al.*, 2001) has to be balanced by the slight cabbage-like off flavour, which needs to be removed before it could attain widespread use.

The heating of betanin causes isomerisation to isobetanin (Schwartz and von Elbe, 1983). During thermal processing of beets there is a loss of colour with some partial regeneration. The likely mechanism for this loss of colour would appear to be the hydrolysis of the Schiff base. This results in a loss of resonance structure for this conjugated cation (Fig. 7.7). The partial restoration of the colour can be explained by the condensation of the hydrolysis products, the amine of cyclodopa-5-0-glycoside and the aldehyde of the betamic acid. This reaction is similar to the first stages given for the Maillard browning reaction (Coulgate, 1988). Melanins may be formed by enzymic browning reactions,



**Fig. 7.19** Thermal degradation products of  $\beta$ -carotene.

including condensation reactions of polyphenols and by non-enzymic browning reactions. Given that in the latter, particularly in the case of caramel, considerable thermal input may be involved in their formation, one would predict that the resultant coloured products should be heat stable.

The thermal stability of the magnesium tetrapyrroles (chlorophyll) and iron tetrapyrroles (myoglobin) differs. This is essentially due to the ease of removal of the  $\text{Mg}^{2+}$  in chlorophyll and the changes in protein conformation due to thermal processing in the latter. Loss of magnesium from the central position within chlorophyll results in pheophytin. The absorption spectrum of this differs from chlorophyll in that the Soret peak at 428 is moved to 408 nm, with a small increase in extinction coefficient and also the peak at 661 nm moves to 667 nm with a marked decrease in extinction coefficient (Houssier and Saver, 1970). Pheophorbide may also be produced by loss of phytol from the pheophytin. This conversion of chlorophyll to pheophytin and pheophorbin is the usual cause for loss of colour during the heat processing of green vegetables.

The effect of heating myoglobin is a denaturation of the protein as measured by extractability of the pigment. The amount of denaturation increases with temperature and it is completely denatured at around 80 to 85°C (Bernofsky *et al.*, 1959). The denatured protein may contain iron in the  $\text{Fe}^{2+}$  (ferrohemochrome) or ferric state (ferrihemochrome). The ferrohemochrome spectra is similar to oxymyoglobin, however, the ferrihemochrome is brown. Denaturation of the globin in myoglobin ( $\text{Fe}^{\text{II}}$ ) using pyridine, a possible model for heat denaturation, results in a high absorption in the Soret region ( $\lambda$  max 419) and particularly at 526 and 557 (Millar, 1994). The visual appearance of cooked meat depends not only on the specific thermal effects on the pigment but on other changes due to the scattering properties of the meat (MacDougall, 1983, MacDougall – Chapter 3 this book).

## 7.11 Irradiation

The primary effects of irradiation on food material is the formation of free radicals and excited molecules (Stewart, 2001). The secondary effect of these is the breakdown of the primary entities (excited molecules, free radicals and ions). Given that the molecular structure common to food colorants is a conjugated diene system, it might be expected that food colorants would be particularly susceptible to the effects of irradiation. Carotene is more sensitive to irradiation than vitamin A when present in foods with high fat content (Diehl and Josephson, 1994). In the dry state  $\beta$  carotene is relatively irradiation stable requiring doses of 20 kGy before inactivation is observed (Lukton and Mackinney, 1956). The observed effects of irradiation on carotenoids in fruit and vegetables is complex and depends on the effects of the irradiation on the ripening process within the fruit and not just the direct effect on the carotenoid (Thomas, 2001). Many of the reported effects of irradiation on anthocyanin content may also be due to the effects of irradiation

on ripening than on anthocyanins *per se* (Thomas, 2001). There are, however, reports of reversible changes in anthocyanins at low irradiation doses (Horubala, 1964, 1968). Since the  $\text{Fe}^{2+}$  in myoglobin can be oxidised it provides additional pathways for the reaction of primary entities of irradiation to those which might be considered for the globin moiety of myoglobin (WHO 1999). Studies in dilute aqueous solutions have shown a complex series of reactions. Ginger *et al.*, (1955) observed the formation of a pink colour in metmyoglobin extracts and formation of metmyoglobin in oxymyoglobin extracts. Tappel (1956) also observed a pink colour when fresh meat was irradiated in a nitrogen atmosphere and postulated it to be oxymyoglobin formed from metmyoglobin via ferrimyoglobin. Several alternative pathways have been proposed (Giddings and Markakis, 1972; Whitburn *et al.*, 1981). In the irradiation of intact meat, rather than aqueous extracts, it has been postulated by Millar *et al.* (2000 a,b) and Moss *et al.* (2000) that the effect on colour may be due to the effect of a secondary product of irradiation, namely carbon monoxide, binding to the iron.

In summary the behaviour of individual food colorants in aqueous solutions or extracts may not predict the colour changes when the food containing them is irradiated. In order to study these we need to consider the interaction of secondary entities of irradiation.

## 7.12 High pressure processing

High pressure processing of food involves the application of hydrostatic pressure typically in the order of 100MPa and above and was first reported in 1899 by Hite. The effects of high pressure for foods are a destruction of microorganisms, denaturation of protein and alteration of enzymatic reactions (Johnston, 1992). From a chemical point of view high pressure will enhance those reactions which give rise to a volume decrease (Hoover *et al.*, 1989). From the chemical structure of the natural food colorants, the one food colorant reaction we might expect to show a volume change is the binding of gaseous ligands with myoglobin. A volume decrease might be postulated as the gaseous ligand moves into the cleft within the myoglobin molecule. The high pressure processing of fruits and vegetables has been reported to result in colour changes, and even changes in extractability of the food colorants. Most of these changes can be attributed to other indirect effects of high pressure on the foods rather than on the colorant *per se*. The research on the effects of high pressure has in the main been on the intact food material, rather than on individual food components and molecular structures (c.f. irradiation, thermal processing).

The high pressure treatment (range 50 to 400MPa) of a purée of persimmon fruit, which are particularly high in  $\beta$  carotene and  $\beta$  cryptoxanthin, was studied by de Ancos *et al.* (2000). Some of the high pressure treatments increased the amount of carotenoids that were extracted and it was suggested that this may be

due to effects of high pressure on the release of carotenoids from protein associates or disruption of the chloroplasts (de Ancos *et al.*, 2000). Zabetakis *et al.* (2000) pressurised strawberries from 200 to 800MPa at a temperature of between 18 and 22°C. From the analysis of the extracted pigments during subsequent storage at 4°C the 800MPa treatment showed the lowest losses (Zabetakis *et al.*, 2000).

The effect of high pressure treatment on myoglobin has been studied both on aqueous solutions (Defaye *et al.*, 1995) and an intact meat (Margey, 2001). From the molecular structure of myoglobin (Fig. 7.5) and known effects of high pressure (Johnston, 1994) there are two effects that might be predicted, (1) the denaturation of the globin portion and (2) binding of ligands, e.g., O<sub>2</sub> to the Fe<sup>2+</sup>. High pressure treatment of solutions of oxymyoglobin at 600MPa (Goutenfongea *et al.*, 1995) and metmyoglobin at 800MPa (Defaye *et al.*, 1995) resulted in denaturation of the molecule. The denatured haem protein has a spectra typical of the heat-denatured myoglobin (Ledward, 1971). There is indication that some of the globin denaturation is reversible (Defaye *et al.*, 1995). In meat the effect of high pressure on the sarcoplasmic proteins needs to be considered in terms of the overall appearance of the meat. Margey *et al.* (1997) suggested that the L\* a\* b\* values of high pressure treated chicken breast were similar to those of cooked chicken breast. Other studies have also indicated increases in lightness when beef, pork and cooked ham were high pressure treated (Goutenfongea *et al.*, 1995). This increase in lightness can be accounted for by increased scattering due to protein denaturation in the meat (MacDougall – Chapter 3). These results show that although we might predict some changes in food colorants from their chemical structure we need to measure the colour changes in the actual food storage/processing condition to determine the complex interactions within the food.

### 7.13 Future trends

Based on current trends one would consider the use of natural food colorants to increase relative to synthetic ones. Also from consumer issues the use of additives in foods except where essential from a food safety aspect (e.g., nitrites in bacon) would also be expected to decrease. Thus the search for other sources of natural food colorants may increase; or it may become beneficial to consider existing food crops selectively bred for higher food colorant contents.

It could also include genetic selection for increasing the ratio of the food colorant molecule with the highest extinction coefficient, or for the anthocyanins the one which may be less pH sensitive. Although such changes could be arrived at sooner through genetic engineering, public opinion at present (at least within the UK) does not seem to fully support genetic engineering, certainly in relation to genetically modified (GM) crops.

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# 8

## Colour stability in vegetables

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### 8.1 Introduction

Colour and colour uniformity are two of the main parameters that define the direct quality of a vegetable. Colour is often taken as an index of freshness, palatability and nutritional value (Haisman and Clarke, 1975). Colour affects the pleasantness and acceptability of a food and interferes with judgements of sweetness, bitterness, saltiness and flavour intensity (Clydesdale, 1993).

The colour of vegetables is due to four principal groups of natural pigments: chlorophylls, carotenoids, flavonoids (e.g. anthocyanins, flavones and flavonols) and betalains (e.g. betacyanins and betaxanthins) (Table 8.1). The chlorophylls are green, the carotenoids yellow, orange or red, the anthocyanins blue or red, the other flavanoids are colourless or yellow and the betalains red or yellow (Table 8.2). In some vegetables, e.g., sweet potatoes and carrots, the yellow and orange colour of carotenoids dominates whereas in green leafy vegetables the

**Table 8.1** Characteristics of natural pigments in vegetables

Pigment group	Approximate no. of compounds	Colour	Sensitive to
Chlorophylls	< 50	Green, olive brown	Heat, acid, alkali, metal cations
Carotenoids	> 300	Yellow, orange, red	Light, oxygen, acid, heat
Anthocyanins	< 150	Red, blue	pH, heat, light, metal cations
Flavonoids	> 600	Yellow	Oxygen, strong acid heat
Betalains	< 100	Red, yellow	Heat, alkali, metal cations

**Table 8.2** Class and colour of natural pigments in vegetables

Pigment class	Structural type	Colour
<i>Chlorophylls and derivatives</i>		
Chlorophylls	Porphyryns	Blue green
Hydroxychlorophylls	Porphyryns	Blue green
Pyrochlorophylls	Porphyryns	Blue green
Chlorophyllides	Porphyryns	Blue green
Pyrochlorophyllides	Porphyryns	Blue green
Pheophytins	Porphyryns	Olive brown
Pyropheophytins	Porphyryns	Olive brown
Hydroxypheophytins	Porphyryns	Olive brown
Pheophorbides	Porphyryns	Olive brown
Pyropheophorbides	Porphyryns	Olive brown
<i>Carotenoids</i>		
Lycopene	Carotene – acyclic	Red
$\beta$ -Carotene	Carotene – bicyclic	Orange
$\alpha$ -Carotene	Carotene – bicyclic	Yellow
$\beta$ -Cryptoxanthin	Xanthophyll – bicyclic	Orange
$\alpha$ -Cryptoxanthin	Xanthophyll – bicyclic	Yellow-orange
Zeaxanthin	Xanthophyll – bicyclic	Yellow-orange
Lutein	Xanthophyll – bicyclic	Yellow
Violaxanthin	Xanthophyll – bicyclic	Yellow
Capsanthin	Xanthophyll – bicyclic	Red
<i>Anthocyanins</i>		
Cyanidins glycosides	Anthocyanidins	Orange-red
Delphinidins glycosides	Anthocyanidins	Blue-red
Malvidins glycosides	Anthocyanidins	Blue-red
Pelargonidins glycosides	Anthocyanidins	Orange
Peonidins glycosides	Anthocyanidins	Orange-red
Petunidins glycosides	Anthocyanidins	Blue-red
<i>Flavonoids</i>		
	Flavones	Yellow
	Flavonols	Yellow
<i>Betalains</i>		
	Betacyanins	Red
	Betaxanthins	Yellow

carotenoids are masked by the green chlorophylls (Table 8.3). In vegetables where the colour of carotenoids dominates, chlorophylls are not developed or they are degraded into colourless products. Perception of vegetable colour is a function of how the vegetable is presented and viewed in relation to the conditions and quality of illumination. The degree of light scattering, absorption and reflection are particularly important. Pigments have a primary role in perceived colour but the physical state of the pigment and the presence and state of non-pigmented substances can have secondary effects such as when the

colour of green vegetables changes from a dull to a bright green colour during blanching (Adams, 1996).

The various conversions in the pigment composition during postharvest storage, processing and freezing are extremely important to food colour. Enzymatic browning caused by oxidative conversion of polyphenols into coloured complexes and non-enzymatic browning is also important to food colour, however, these processes are not included here.

## **8.2 The chemistry and occurrence of vegetable pigments: chlorophylls, carotenoids, flavonoids and betalains**

### **8.2.1 Chlorophylls**

The green chlorophylls *a* and *b* are ubiquitous in all edible parts of vegetables, whether it is roots, stems, leaves, flowers, fruits, or seeds, at least at a certain developmental stage (Table 8.3). Certainly, chlorophylls are found in higher amounts in leaf vegetables and in lower amounts in immature fruits or inflorescence vegetables. Chlorophylls are porphyrins containing the basic tetrapyrrole ring, of which one is reduced. The four rings are co-ordinated with a magnesium ion ( $Mg^{2+}$ ). A fifth isocyclic ring is found near the third pyrrole ring. At the fourth ring, the propionic acid substituent is esterified with the hydrophobic diterpene alcohol phytol (Fig. 8.1). All green plants contain chlorophyll *a* and *b*. In higher plants, chlorophyll *a* to *b* is present in an approximately 3 to 1 ratio, however, the ratio varies with growth conditions, environment and development stage. Plant species exposed to sun tend to have higher ratio (~ 3.2 to 4.0) than shade plants (~ 2.6 to 3.2) (Lichtenthaler, 1971; Lichtenthaler *et al.*, 1981). Chlorophyll *a* is more rapidly degraded than chlorophyll *b*, thus the ratio is continuously shifted to lower values during leaf senescence (Gross, 1991).

The properties of chlorophylls in green tissues depend on the nature of its association with lipoproteins of the chloroplast (López-Ayerra *et al.*, 1998). Disruption of the chlorophyll structure cannot take place until the membrane array of the chlorophyll has been disorganised (Haisman and Clarke, 1975). The degradation mechanisms during ripening and senescence, although extremely important to food colour, is largely unknown, whereas it is clearer in food processing (Clydesdale, 1993).

### **8.2.2 Carotenoids**

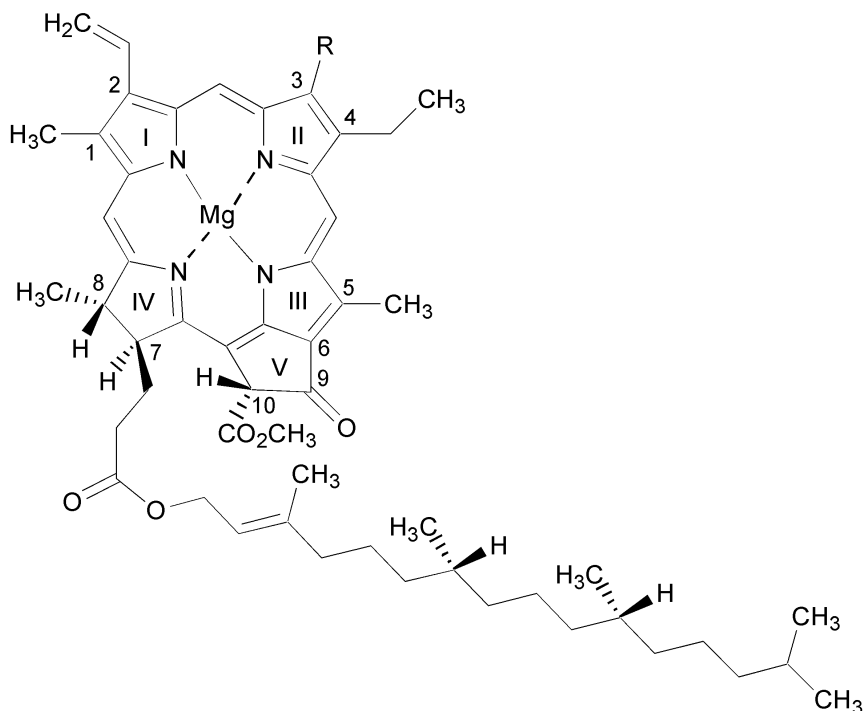
The carotenoids are a group of mainly lipid-soluble compounds, which are formed by joining eight isoprenoid units. The carotenoids are divided into two groups: the carotenes that contain only carbon and hydrogen atoms, resulting in low polarity, and the xanthophylls, which are the oxygenated derivatives (Fig. 8.2). The xanthophylls contain at least one oxygen, e.g., a keto, hydroxy or epoxy group, resulting in more polar compounds (Gross, 1991; van der Berg *et al.*, 2000).

**Table 8.3** Principal colour and pigments of vegetables with diverse morphological structures.

Edible part	Common name	Latin name	Principal colour	Principal pigment group responsible for the colour
Root	Beet	<i>Beta vulgaris</i>	Red, yellow or white	Betalains
	Carrot	<i>Daucus carota</i>	Orange, yellow, red	Carotenoids
	Radish	<i>Raphanus sativus</i>	Red, white or green outside – white inside	Anthocyanins, flavonoids
	Scorzonera	<i>Scorzonera hispanica</i>	Brown outside – white inside	1
	Parsnip	<i>Pastinaca sativa</i>	White inside	1
	Celeriac	<i>Apium graveolens</i>	Brown outside – white inside	1
	Sweet potato	<i>Ipomoea batatas</i>	Purple, white outside, orange, yellow, white, purple inside	Carotenoids, anthocyanins, flavonoids
Stem	Asparagus	<i>Asparagus officinalis</i>	White or green	Chlorophylls
	Potato	<i>Solanum tuberosum</i>	Red, yellow, white, green or purple outside – white inside	Chlorophylls, anthocyanins, flavonoids
Leaf	Jerusalem artichoke	<i>Helianthus tuberosus</i>	Brown outside – white inside	1
	Onion	<i>Allium cepa</i>	White, red	Anthocyanins, flavonoids
	Leek	<i>Allium porrum</i>	White-green	Chlorophylls
	Parsley	<i>Petroselinum crispum</i>	Green	Chlorophylls
	Fennel	<i>Foeniculum vulgare</i>	White-green	Chlorophylls
	Lettuce	<i>Lactuca sativa</i>	Green	Chlorophylls
	Leaf celery	<i>Apium graveolens</i> var. <i>dulce</i>	Green	Chlorophylls
	Chicory	<i>Cichorium intybus</i>	Green or white	Chlorophylls

Cabbage	<i>Brassica oleracea</i> var. <i>capitata</i>	Green or red	Chlorophylls, anthocyanins
Chinese cabbage	<i>Brassica pekinensis</i>	Green	Chlorophylls
Spinach	<i>Spinacia oleracea</i>	Green	Chlorophylls
Kale	<i>Brassica oleracea</i> var. <i>acephala</i>	Green	Chlorophylls
Brussels sprouts	<i>Brassica oleracea</i> var. <i>gemmifera</i>	Green	Chlorophylls
Immature flower bud	<i>Brassica oleracea</i> var. <i>botrytis</i>	White	<sup>1</sup>
Cauliflower			
Broccoli	<i>Brassica oleracea</i> var. <i>italica</i>	Green, purple	Chlorophylls, anthocyanins
Green peas	<i>Pisum sativum</i>	Green	Chlorophylls
Green beans	<i>Phaseolus vulgaris</i>	Green	Chlorophylls
Pumpkin, squash	<i>Cucurbita pepo</i> , <i>C. moschata</i> , <i>C. maxima</i>	White, yellow, orange, green	Carotenoids, chlorophylls
Cucumber	<i>Cucumis sativus</i>	Green	Chlorophylls
Eggplant	<i>Solanum melongena</i>	Purple	Anthocyanins, flavonoids
Maize	<i>Zea mays</i>	Yellow, orange, purple	Carotenoids, anthocyanins
Tomato	<i>Lycopersicon esculentum</i>	Red	Carotenoids
Pepper	<i>Capsicum annuum</i>	Yellow, red, green	Carotenoids, chlorophylls

<sup>1</sup>Chloroplasts and/or chromoplasts not developed.

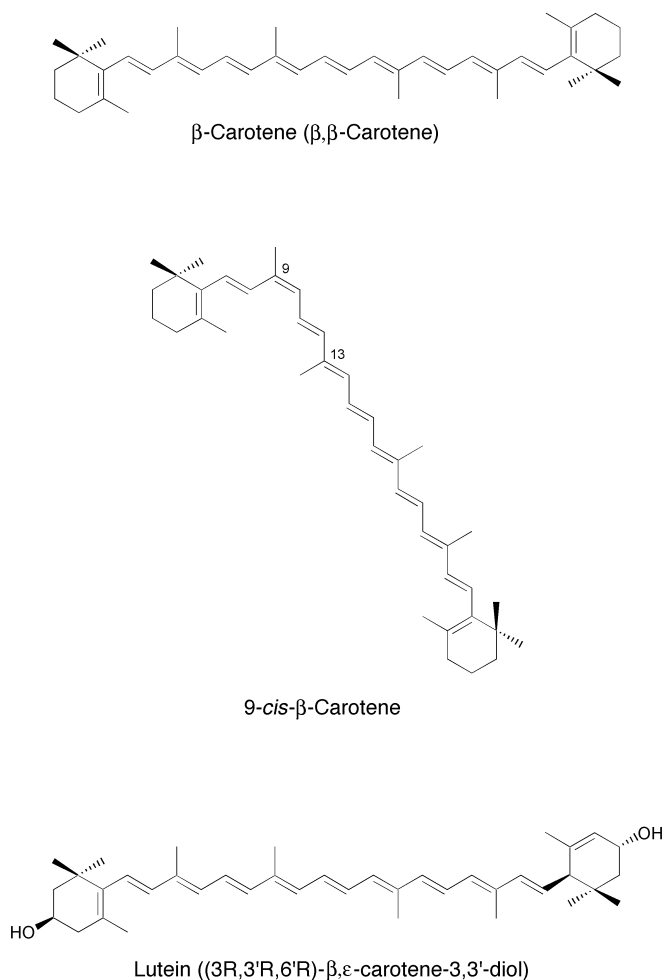


**Chlorophyll *a***, R = CH<sub>3</sub>

**Chlorophyll *b***, R = CHO

**Fig. 8.1** Structure of chlorophyll *a* and *b*.

The yellow, orange and red colour of the carotenoids is due to a system of conjugated carbon-carbon double bonds. At least seven double bonds in the conjugated system are necessary to have an impact on colour. Extension of the conjugated system gives a deeper colour. The intensity and hues of the colours depend on the type of the carotenoid, their concentrations and their physical state (Table 8.2) (Purcell *et al.*, 1969; Carnevale *et al.*, 1980; Rodriguez-Amaya, 1997). The contents of carotenoids or carotenes are generally correlated to colour of yellow, orange and red vegetables, e.g., carrots and tomatoes (Table 8.3) (Carnevale *et al.*, 1980; Tantchev *et al.*, 1997; Arias *et al.*, 2000). Estimations of the carotenoid content in vegetables have been carried out using objective colour measurements. In carrot juice, a high correlation was found between total carotene content and colour measured by tristimulus colorimetry (Munsch *et al.*, 1983). The lycopene content in six mature groups of tomatoes, varying in colour, correlated with surface colour measured by a Minolta chroma meter and expressed as  $a^*/b^*$ .  $+a^*$  value expresses redness and  $-a^*$  greenness.  $+b^*$  expresses yellowness and  $-b^*$  blueness (Arias *et al.*, 2000). In white-



**Fig. 8.2** Structure of  $\beta$ -carotene, a *cis*-isomer of  $\beta$ -carotene and lutein.

fleshed sweet potatoes, the  $b^*$  value gave a good estimation of the  $\beta$ -carotene content (Ameny and Wilson, 1997).

In green leafy vegetables, chlorophylls and carotenoids are located together in chlorophyll-carotenoid-protein complexes. In photosynthesising tissues, the carotenoids act as accessory pigments in light harvesting in the photosynthesis and prevention of photooxidative damage (Gross, 1991; Bartley and Scolnik, 1995; Demmig-Adams *et al.*, 1996).  $\beta$ -Carotene is the major carotenoid in green leafy vegetables (Fig. 8.2). Spinach and green kale, which are commonly eaten in the western world, have high contents. The yellow and orange colours of the carotenoids in green vegetables are masked by the green colour of the chlorophylls, and thus the content of carotenoids cannot be related to the colour of these vegetables (Table 8.3). In other vegetables, the carotenoids are located



within the carotenoid-containing plastids, the chromoplasts, e.g., in roots and fruit vegetables. The composition and the concentration of carotenoids vary between species of vegetables (Table 8.3). Roots with a high content of total carotenoids and/or carotenes are carrots (*Daucus carota*), dominated by  $\alpha$ - and  $\beta$ -carotene and orange-coloured sweet potatoes (*Imomoea batatas*). Important fruit vegetables pigmented with carotenoids are tomatoes (*Lycopersicon esculentum*), which are dominated by the red pigment, lycopene, pepper fruits (*Capsicum annuum*), with capsanthin as the most abundant carotenoid, maize (*Zea mays*), squashes and pumpkins (*Cucurbita* spp.) (Table 8.3) (Gross, 1991).

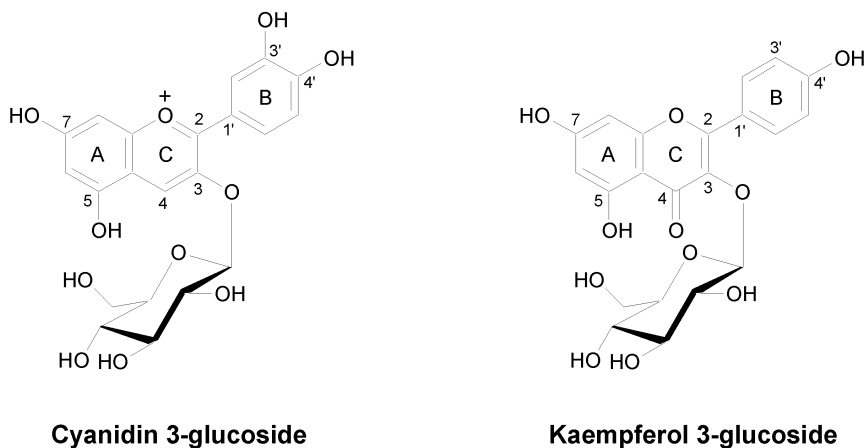
### 8.2.3 Flavonoids and betalains

#### *Anthocyanins and other flavonoids*

Anthocyanins, a class of flavonoids, are responsible for the red and purple colours associated with many fruits and vegetables. Anthocyanins occur in epidermal and subepidermal cells, dissolved in vacuoles or accumulated in vesicles called anthocyanoplasts. In general, the anthocyanin content in most fruits and vegetables varies between 0.1 and 1% of the dry matter content (Swain and Bate-Smith, 1962).

Anthocyanins are composed of an aglycone (anthocyanidin), sugar, and sometimes phenolic and/or minor organic acids (Fig. 8.3). Twenty-two aglycones are known, of which 18 occur naturally. Pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin are the most abundant anthocyanins in vegetables. Free aglycones occur very rarely in plants, because the glycosylated form is more stable. The sugars are represented by one or more units of glucose (most common), rhamnose, galactose, arabinose, xylose or glucuronic acid connected to the aglycone most frequently in the 3-OH, 5-OH or 7-OH positions. Anthocyanins may be acylated with one or more molecules of phenolic acids such as *p*-coumaric acid, ferulic acid, cinnamic acid and caffeic acid or aliphatic acids, e.g., malonic acid and acetic acid (Mazza and Brouillard, 1987).

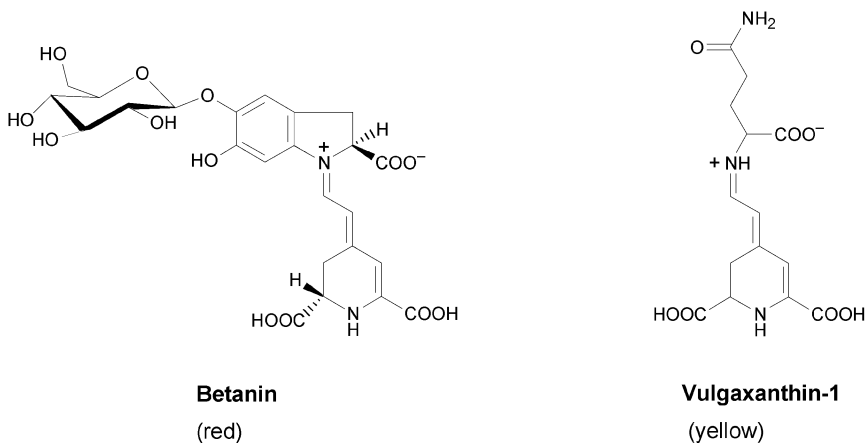
Plants that contain anthocyanins also invariably contain the yellow or colourless flavonoids. Flavonoids are localised in epidermal cells and more than 4000 different structures have been found (Harborne and Grayer, 1988). The most frequently found flavonoid structures in plants are based on the flavonol and flavone skeletons, which can be hydroxylated, methoxylated or glycosylated in various positions as for the anthocyanins. A simple flavonol glucoside, kaempferol 3-*O*- $\beta$ -glucoside is shown in Fig. 8.3. Anthocyanins are widespread in food plants, occurring in 27 families (Timberlake, 1988). The occurrence of anthocyanins and other flavonoids in vegetables has been reviewed by Francis (1989), Clifford (2000), Clydesdale (2000), Ames and Hofmann (2001) and Harborne and Williams (2001). New data on anthocyanins and other flavonoids in vegetables includes about eleven food plants: spinach (Gil *et al.*, 1999), red cabbage (Idaka *et al.*, 1987; Ikeda *et al.*, 1987; Nakatani *et al.*, 1987), cabbage (Nielsen *et al.*, 1993 and 1998; Price *et al.*, 1998, Lugasi *et al.*, 1999), red radish



**Fig. 8.3** Structure of cyanidin 3-glucoside and kaempferol 3-glucoside.

(Giusti and Wrolstad, 1996; Giusti *et al.*, 1998), carrot (Hopp and Seitz 1987; Dougall *et al.*, 1998), sweet potato (Otake *et al.*, 1992; Goda *et al.*, 1997; Lewis *et al.*, 1998; Rodriguez-Saona *et al.*, 1998; Terahara *et al.*, 1999), rhubarb (Agarwal *et al.*, 2001), olives (Romani *et al.*, 1999 and 2000), purple pods of pea (Terahara *et al.*, 2000), onion (Andersen and Fossen, 1995; Fossen *et al.*, 1996; Donner *et al.*, 1998), and eggplants (Bajaj *et al.*, 1990; Kaneyuki *et al.*, 1999).

White cabbage is rich in flavonol glucosides, where acylated and unacylated tri- and tetraglucosides of kaempferol and quercetin have been isolated and identified (Nielsen *et al.*, 1993; 1998). In broccoli florets, the main flavonol glycosides were identified as sophorosides of quercetin and kaempferol, but all the flavonoids have not yet been determined. The flavonols were present in raw florets at 65 mg kg<sup>-1</sup> and 166 mg kg<sup>-1</sup> fresh weight, respectively (Price *et al.*, 1998). Red radish is rich in phenolic compounds (0.233 mg kg<sup>-1</sup> fresh weight), 50% of which are acylated anthocyanins (mainly pelargonidin derivatives). The remaining compounds are phenolic acids or esters whereas no flavonols have been detected (Malien-Aubert *et al.*, 2001). In purple carrot, the main pigments are cyanidin glycosylated with various sugar moieties and occasionally acylated with ferulic acid (Hopp and Seitz, 1987; Harborne and Grayer, 1988; Dougall *et al.*, 1998). Cyanidin 3-glucoside and cyanidin 3-rutinoside have been identified from an olive extract (Romani *et al.*, 1999; Romani *et al.*, 2000). From purple pods of pea, an acylated anthocyanin, delphinidin 3-xylosylgalactoside-5-acetylglucoside has been isolated together with its deacylated form (Terahara *et al.*, 2000). In onions acylated anthocyanins have recently been found, but with an unusual acylation of the 3-position in the sugar moiety (Andersen and Fossen, 1995). Eggplant epidermis is rich in polyphenolic components such as nasunin (purple) and hyacin (blue). Nasunin is an anthocyanin with a potent scavenge potential against superoxide anion radicals and hydroxyl radicals (Bajaj *et al.*, 1990; Kaneyuki *et al.*, 1999).



**Fig. 8.4** Structure of betanin and vulgaxanthin-1.

### *Betalains*

The betalain group contains approximately 50 red pigments termed betacyanins and 20 yellow pigments termed betaxanthins. Depending on resonance structures, the pigments can be red as in betacyanin, e.g., betanin, or yellow as in vulgaxanthin-1 (Fig. 8.4) (Strack *et al.*, 1993). Betacyanins are found most frequently as betanidin or isobetanidin combined with glucose, which may be acylated. Betalains are characteristic pigments in plant members of the Carophyllales. The only plant foods containing betacyanins are red beet (*Beta vulgaris*), chard (*Beta vulgaris*), cactus fruit (*Opuntia ficus-indica*), and pokeberries (*Phytolacca esculenta*). The berries of pokeberries are not eaten, however, leaves can be used as a green vegetable (Adams, 1981).

The anthocyanin-flavonoid and betacyanin-betaxanthin groups are not mutually present in the same plants, and therefore the presence of betalains excludes the presence of anthocyanins and vice versa (Mabry *et al.*, 1963). In most cases it is impossible to distinguish between anthocyanins and betalains visually, but tests have been developed to distinguish between the colour of these pigments at different pH and temperature (Strack *et al.*, 1993).

## 8.3 The stability of pigments

### 8.3.1 Chlorophylls

Chlorophylls are green in colour because they absorb strongly in the red and blue regions of the visible spectrum. Chlorophylls *a* and *b* differ in structure only by the presence of a methyl and aldehyde group (CHO), respectively, at position 3 (Fig. 8.1). Chlorophylls *a'* and *b'* are 10-epichlorophylls related to the parent pigments by inversion of the C-10 carbomethoxy group ( $-\text{CO}_2\text{CH}_3$ ) and have the same absorption spectra as their parent pigments. Chlorophyll *a'*

and  $b'$  are interconvertible with their parent pigments, a process that occurs slowly at room temperature but rapidly in heated plant tissues. Bacon and Holden (1967) showed that 10% of chlorophylls  $a$  and  $b$  were converted to chlorophylls  $a'$  and  $b'$ , respectively, when leaves were held in boiling water for five minutes. Also, deep-frozen plant material, such as spinach, contains significant amounts of chlorophylls  $a'$  and  $b'$  (Scholz and Ballschmiter, 1981). The conversion of chlorophyll  $a$  and  $b$  to the respective epimers also occurs rapidly in organic solvents. Consequently, the two epimers have often been considered artefacts produced in the course of handling plant extracts. Chlorophyll  $a'$  is, however, a natural constituent of the photosynthetic membrane (Watanabe *et al.*, 1985).

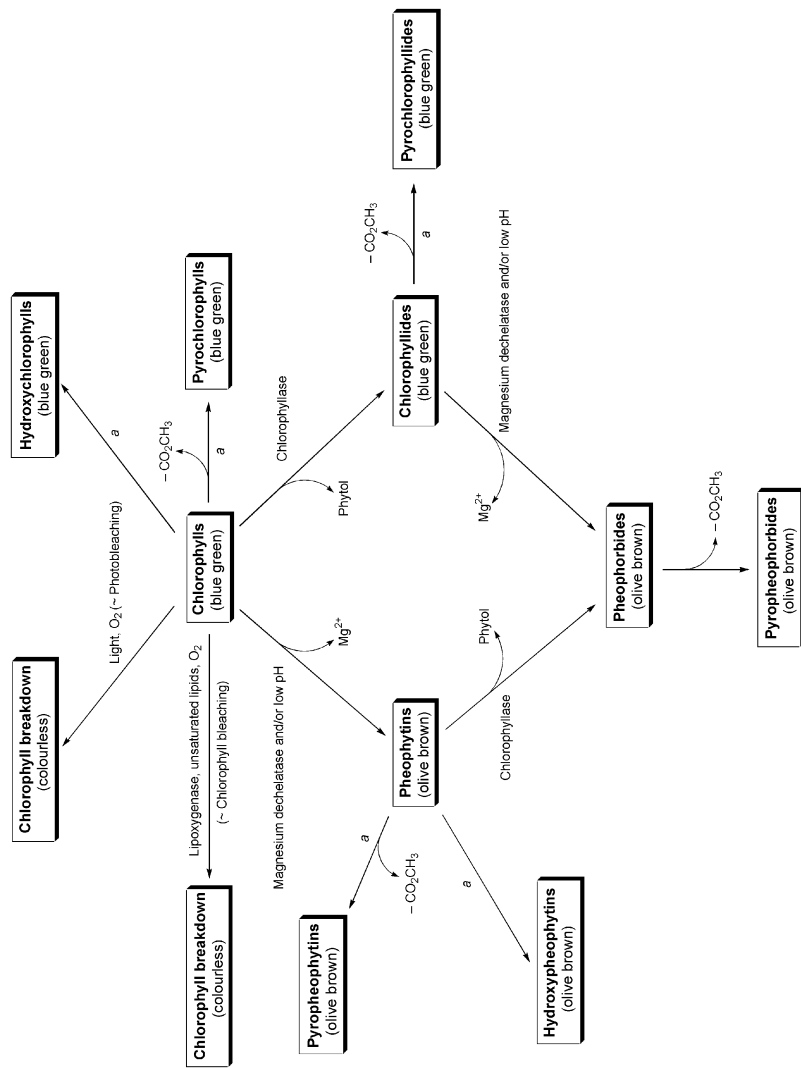
Chlorophylls are relatively unstable in green vegetables subjected to various processing and storage conditions. Chlorophylls undergo distinct types of degradation leading to changes or loss of colour that are similar to that occurring in senescing plants. As consumers perceive the green colour of chlorophyll as an indicator for 'freshness' of food, many studies have been carried out to understand and control the degradation of chlorophylls.

Chlorophylls are readily transformed or degraded, both *in vivo* and *in vitro*, into a series of derivatives with olive-brown and green colours or may simply be degraded to fluorescent or non-fluorescent colourless substances due to chlorophyll bleaching or photobleaching. The olive-brown degradation products of chlorophylls are magnesium-free derivatives and include the pheophytins, pheophorbides, pyropheophytins and pyropheophorbides. The green coloured degradation products include the chlorophyllides, pyrochlorophylls and the allomerized chlorophylls (hydroxychlorophylls) which still contain magnesium and the metallocomplexes where the magnesium ion has been replaced by, e.g., zinc (II) or copper (II) ions. A proposed mechanism for the degradation of chlorophylls in green vegetables is given in Fig. 8.5.

### *Chlorophyllides*

The phytol ester group of chlorophylls can easily be hydrolysed to give chlorophyllide and phytol. The hydrolysis can take place under mild conditions either by acid or alkali. However, in general the chlorophyllides are prepared enzymatically, the hydrolysis being catalysed by the enzyme chlorophyllase, which seems to be present in all green plant tissues, being higher in leaves and lower in roots and seeds. Furthermore, its activity varies with the plant species. Sugar beet (*Beta vulgaris*) or common cocklebur (*Xanthium pennsylvanicum*) are examples of plant species whose leaves are especially rich in chlorophyllase (Holden, 1961; Pennington *et al.*, 1964).

Chlorophyllase is an intrinsic membrane glycoprotein situated in the thylakoid membrane (Terpstra, 1981). It does not normally interact with its chlorophyll substrate, although chlorophyll is situated in the same membrane. In intact plant tissue, chlorophyllase is kept in an inactive and stable conformation by the adjacent lipids of the thylakoid membrane (Lambers and Terpstra, 1985). In order to activate chlorophyllase, a substantial disruption of the thylakoid membrane has to



**Fig. 8.5** A proposed mechanism for the degradation of chlorophylls in processed and stored vegetables. a = induced by heat.

occur. Consequently, the formation of chlorophyllides in fresh plant tissue does not occur until the enzyme has been activated through heat, mechanical damage or other kinds of disruption. Optimum temperatures for heat activation of chlorophyllase in vegetables range from 60 to 82°C whereas blanching at 100°C inactivates the enzyme (Weast and Mackinney, 1940; Jones *et al.*, 1963; Loef and Thung, 1965; Clydesdale and Francis, 1968; Ogura *et al.*, 1987).

#### *Pheophytins*

Pheophytins are the magnesium-free derivatives of the chlorophylls. The most common mechanisms of chlorophyll *a* and *b* degradation seem to be the acid-catalysed transformation into pheophytin *a* and *b*, respectively, which is accomplished by cellular acids. In this process the magnesium ion of the chlorophylls is displaced with two hydrogens leading to the respective pheophytins. The removal of the central magnesium ion in chlorophylls may also be accomplished by the enzyme magnesium dechelataase. The plant material changes colour from blue-green to dull olive-green by conversion of chlorophylls *a* and *b* to their respective pheophytins. This alteration is most widespread in green vegetables and takes place during thermal processing, freezing preservation, and storage.

#### *Pheophorbides*

Pheophorbides are hydrolysed chlorophylls without phytol that have lost the magnesium ion. Pheophorbides can either be produced by enzymatic hydrolysis of pheophytins, a process that is catalysed by chlorophyllase, or by removal of the central magnesium ion from chlorophyllides, which is accomplished by, e.g., cellular acids or the enzyme magnesium dechelataase (Fig. 8.5). Pheophorbides have been shown to be the predominant pigments in brined cucumbers suggesting that brining treatment favours chlorophyllase activity (Jones *et al.*, 1961, 1962; White *et al.*, 1963) and that the pH of the brine medium furthermore favours the replacement of magnesium in the chlorophylls and chlorophyllides by hydrogen.

#### *Pyrochlorophylls, pyrochlorophyllides, pyropheophytins and pyropheophorbides*

Pyroderivatives of chlorophylls or their derivatives are the compounds that have lost the carbomethoxy group ( $-\text{CO}_2\text{CH}_3$ ) at C-10 of the isocyclic ring, the group being replaced by hydrogen. Pyropheophytins *a* and *b* have for example been reported from spinach purée heated between 116 and 126°C (Schwartz and von Elbe, 1983) and recent findings indicate that pyropheophytins *a* and *b* are the major chlorophyll derivatives responsible for the olive-green colour in canned vegetables (Gross, 1991).

#### *Allomerised chlorophylls (hydroxychlorophylls)*

Hydroxychlorophylls are oxidized chlorophylls with the hydroxy group being located at C-10. The hydroxychlorophylls of chlorophylls *a* and *b* are called

chlorophylls *a-1* and *b-1*, respectively. Oxidation of chlorophylls to hydroxy-chlorophylls has recently been proved to proceed via a free-radical mechanism, the autooxidation being inhibited by carotenoids (Hynninen, 1981). Hydroxychlorophylls may be formed during boiling of green plant tissues and have also been detected in rehydrated spinach together with hydroxypheophytins (Gauthier-Jaques *et al.*, 2001).

#### *Metallocomplexes*

Formation of green metallocomplexes of chlorophyll derivatives during thermal processing, also known as 'regreening', is considered to be a promising method to preserve the colour of canned green vegetables. Zinc and copper metals are introduced into the chlorophyll porphyrin ring. The metal chlorophyll derivatives form a firm bond that is more resistant to acid and heat than naturally occurring magnesium chlorophyll complexes (Humphrey, 1980).

#### *Chlorophyll bleaching*

The bleaching of chlorophyll seems to be an oxidative process involving enzymes such as lipoxygenase, peroxidases, and oxidases, of which the former enzyme seems to be the main oxidative catalyst in vegetables (Gross, 1991). The bleaching of chlorophylls during fat peroxidation is very common in vegetables that have not been subjected to heat. The process of fat peroxidation starts with the accumulation of fatty acids in plant membranes after exposure of plants to environmental stress, e.g., freezing, or during senescence. This is a consequence of the selective enzymatic degradation of phospholipids by phospholipases (Barclay and McKersie, 1994), or by lipolytic acylhydrolase, since the activity of these enzymes increases with senescence. The resulting free fatty acids may be oxidised by lipoxygenase to form hydroperoxides (Whitaker, 1990) which can then react with other constituents such as vitamins, carotenoids and stimulate oxidative degradation of chlorophyll to colourless compounds (Yamauchi and Watada, 1991).

#### *Photobleaching*

Chlorophylls are sensitive to light, and photobleaching is one of the most important mechanisms for chlorophyll breakdown. Photobleaching does not occur *in vivo* as long as chlorophylls are found in intact thylakoid membranes and photoprotected by the endogenous carotenoids. *In vitro*, chlorophyll solutions are irreversibly bleached by light in the presence of oxygen, indicating that photobleaching of chlorophyll is a photooxidative degradation of chlorophyll (Jen and Mackinney, 1970a and b). The bleaching of cucumber (*Cucumis sativus*) leaves which occurs only in light with an optimum temperature around 1–5°C (van Hasselt and Strikwerda, 1976) is an example of a photooxidative degradation of chlorophyll. Wise and Naylor (1987) have given evidence for the role of reactive oxygen species such as singlet oxygen ( $^1\text{O}_2$ ) and superoxide ( $\text{O}_2^-$ ) in the breakdown of chlorophyll during photobleaching.

### 8.3.2 The carotenoids

#### *Oxidation*

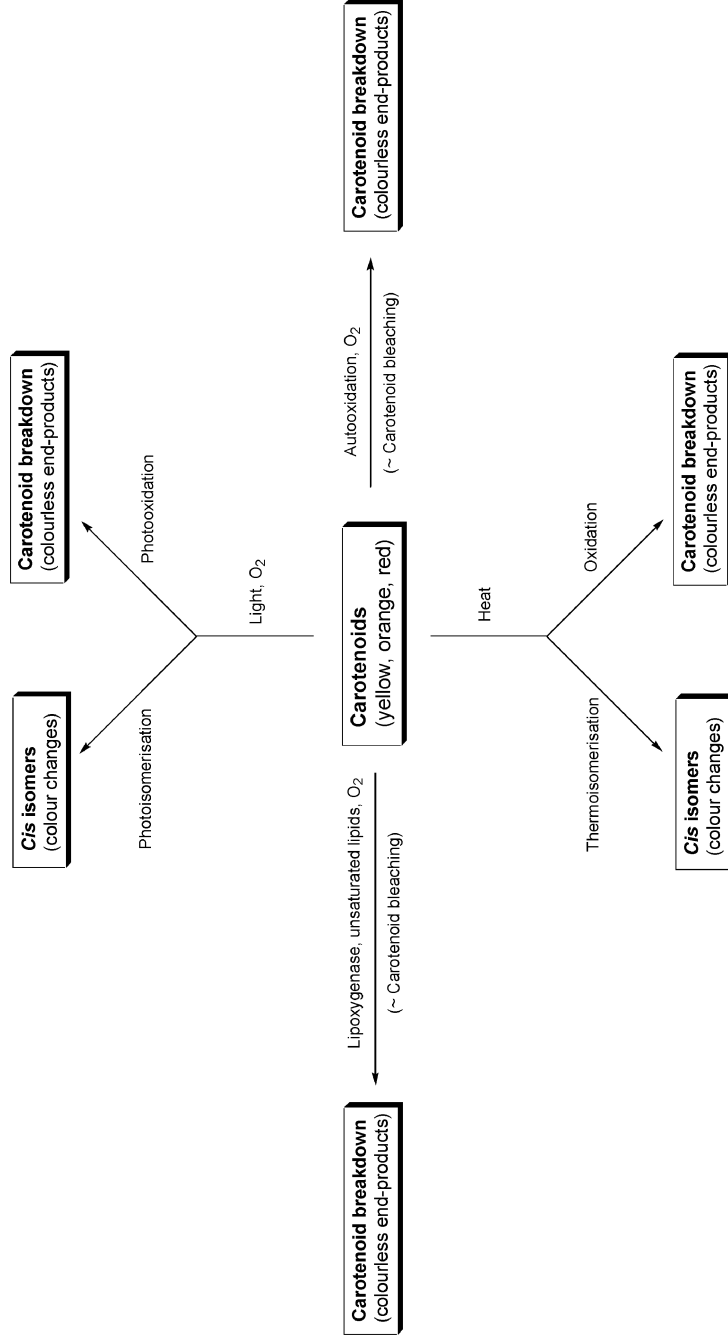
The major cause of carotenoid losses in vegetables is due to oxidation of the highly unsaturated carotenoid structure (Fig. 8.2). Oxidation may occur by (a) autooxidation, which is a spontaneous, free-radical chain reaction in the presence of oxygen, (b) photooxidation produced by oxygen in the presence of light and (c) coupled oxidation in lipid containing systems (Fig. 8.6) (Goldman *et al.*, 1983; Gross, 1991). These oxidation reactions may result in carotene bleaching, which is a consequence of formation of colourless end-products. Colour bleaching leads to changes or loss of the colour. The severity of oxidation depends on the structure of the carotenoids and the environmental conditions, and the compounds being formed depend on the oxidation process and the carotenoid structure (Ramakrishnan and Francis, 1980).

#### *Autooxidation*

During autooxidation of the carotenoids, alkylperoxyl radicals are formed. These radicals mainly attack the double bonds resulting in formation of epoxides. Autooxidation of  $\beta$ -carotene has been studied in model systems. The products being formed during autooxidation of  $\beta$ -carotene were identified as a mixture of products with epoxy, hydroxy and carbonyl groups (Packer *et al.*, 1981; Mordí *et al.*, 1991; Liebler and Kennedy, 1992; Liebler, 1993; Yanishlieva *et al.*, 1998).

The rate of autooxidation between carotenoids and free oxygen radicals depends for example on the structure of the carotenoids, available oxygen, temperature, light, water activity, pH, and the presence of pro- and antioxidants (Chou and Breene, 1972; Ramakrishnan and Francis, 1980; Carnevale *et al.*, 1980; Goldman *et al.*, 1983; Gross, 1991; Minguez-Mosquera and Jaren-Galan, 1995; Yanishlieva *et al.*, 1998). If one or more  $\beta$ -ionine rings are present in the carotenoid structure, the rate of autooxidation seems to depend on their polarity. The most apolar carotenoids, e.g.,  $\beta$ -carotene, are more susceptible to autooxidation than the polar xanthophylls, e.g., zeaxanthin, containing two hydroxy groups (Ramakrishnan and Francis, 1980). Goldman *et al.* (1983) reported that the rate of decoloration of  $\beta$ -carotene was influenced by the amount of oxygen absorbed. In dried tomato products, very low water activity leads to fading and discolouration probably as a consequence of autooxidation of lycopene. The presence of water and high water activity, however, seem to inhibit discoloration of  $\beta$ -carotene (Chou and Breene, 1972; Bošković, 1979; Goldman *et al.*, 1983). Enhanced temperature increases the  $\beta$ -carotene oxidation. At temperatures above 170°C,  $\beta$ -carotene may be fragmented to polar short-chain hydrocarbons and/or longer-chain aromatic hydrocarbons, e.g., ionene (Biacs and Wissgott, 1997). Volatile compounds, which predominately were formed by thermal degradation of  $\beta$ -carotene at 190–220°C in the presence of air, were  $\beta$ -ionone, dihydroactinidiolide and 5,6-epoxy- $\beta$ -ionone (Schreier *et al.*, 1979). The presence of pro- or antioxidants affects the rate of autooxidation. Addition of unsaturated lipids results in increased  $\beta$ -carotene degradation. The





**Fig. 8.6** A proposed mechanism for the degradation and isomerisation of carotenoids in processed and stored vegetables.

pro-oxidative effect of the lipids increases with the degree of unsaturation (Budowsky and Bondi, 1960; Gross, 1991).

#### *Photooxidation*

Carotenoids are sensitive to light and may be subjected to photooxidation in the presence of oxygen. The rate of decolouration of the carotenoids by photooxidation is enhanced by the presence of sensitisers and the intensity of illumination by fluorescent light (Carnevale *et al.*, 1980; Gross, 1991; Yanishlieva *et al.*, 1998; Lennersten and Lingnert, 2000). In the photosensitised oxidation, an excited sensitiser produces singlet oxygen ( $^1\text{O}_2$ ), which can be quenched by the carotenoids. In general, photooxidation of  $\beta$ -carotene results in formation of *cis*-isomers of  $\beta$ -carotene and its 5,6- and 5,8-epoxides. Other products being formed are compounds such as aurochrome and mutatochrome (Zinsou and Costes, 1973; Gross, 1991; Biacs and Wissgott, 1997; Yanishlieva *et al.*, 1998).

#### *Coupled oxidation*

The carotenoids may undergo coupled oxidation in a lipid system, which also leads to carotenoid bleaching (Oszmianski and Lee, 1990). The role of  $\beta$ -carotene in lipid oxidation has been extensively investigated in oils and fats (Yanishlieva *et al.*, 1998). Coupled oxidation of  $\beta$ -carotene is associated with the linoleate-lipoxygenase system in raw vegetables that have been exposed to stress. The fat peroxidation and the following formation of peroxides in the presence of lipoxygenase are described in Section 8.3.1. The peroxides being formed oxidise the carotenoids by a secondary or a coupled reaction, resulting in decolouration of the carotenoids (Park, 1987; Oszmianski and Lee, 1990; Gross, 1991). Lipoxygenases are found in different vegetable crops, e.g., peas, alfalfa, broad bean, soybean, potato, cauliflowers, corn and eggplants. However, carrots do not contain lipoxygenase (Eskin *et al.*, 1977; Vora *et al.*, 1999). Other factors that affect the rate of coupled oxidation of the carotenoids in lipid systems are available oxygen and the presence of antioxidants, e.g., phenolics (Oszmianski and Lee, 1990). The products obtained by oxidation of  $\beta$ -carotene in a linolate-lipoxygenase system are identified as *cis* isomers of  $\beta$ -carotene,  $\beta$ -carotene monoepoxide, aurochrome, and several conjugated ketones (Friend, 1958; Gross, 1991). Volatile products being formed during bleaching with soybean lipoxygenase are  $\beta$ -ionone,  $\beta$ -ionone epoxide and dihydroactinidiolide (Grosch *et al.*, 1977; Gross, 1991).

#### *Cis-trans-isomerisation*

As described above, different *cis*-isomers are formed during oxidation. The carotenoid structure makes a *cis-trans*-isomerisation possible around the double bonds (Fig. 8.2). In vegetables, the carotenoids are predominantly the all-*trans* isomers, which may be converted to the *cis*-isomers. *Cis-trans* isomerisation results in colour changes in vegetable products as the spectral properties of the *cis*-carotenoids are different from the corresponding *trans*-carotenoids. Insertion of one or more *cis*-double bonds in an all-*trans* conjugated system results in a

hypsochromic shift of 2 to 5 nm. A subsidiary peak is also present in the near-ultraviolet absorption spectrum, if a *cis*-isomer is present. These different spectral properties of *cis*-isomers result in a lighter hue of lower intensity compared to the corresponding *trans*-isomers (Simpson, 1985; Gross, 1991). In dehydrated tomato products, these colour changes were observed as a decrease in the red colour intensity as a result of *cis*-isomerisation of all-*trans*-lycopene (Bošković, 1979; Biacs and Wissgott, 1997; Rodriguez-Amaya, 1997).

Acids, light and heating promote isomerisation of all-*trans*-carotenoids to *cis*-forms (Park, 1987; Chen *et al.*, 1994; Biacs and Wissgott, 1997; Rodriguez-Amaya, 1997; Mortensen and Skibsted, 2000). In general, the rate of *cis*-isomerisation increases with enhanced temperatures. Heating of tomatoes at 100°C for 30 minutes resulted in conversion of all-*trans*- $\beta$ -carotene and all-*trans*-lutein to the *cis* configurations. However, the isomerisation depends on the structure of the carotenoids. Especially the epoxycarotenoids are sensitive to heat treatment, whereas lutein and carotenes, e.g., lycopene,  $\alpha$ - and  $\beta$ -carotene, survive the heat treatment (Khachik *et al.*, 1992; Nguyen *et al.*, 2001). Chen *et al.* (1994) studied thermoisomerisation of  $\alpha$ - and  $\beta$ -carotene in a model system. The concentration of all-*trans*- $\alpha$ -carotene and all-*trans*- $\beta$ -carotene was only slightly affected at 50°C or 100°C for 30 minutes. At 150°C all-*trans*- $\beta$ -carotene was isomerised, 9-*cis*- and 13-*cis*- $\beta$ -carotene were the major isomers being formed. Isomerisation of all-*trans*- $\alpha$ -carotene resulted in formation of 13-*cis*- $\alpha$ -carotene in the highest concentration.

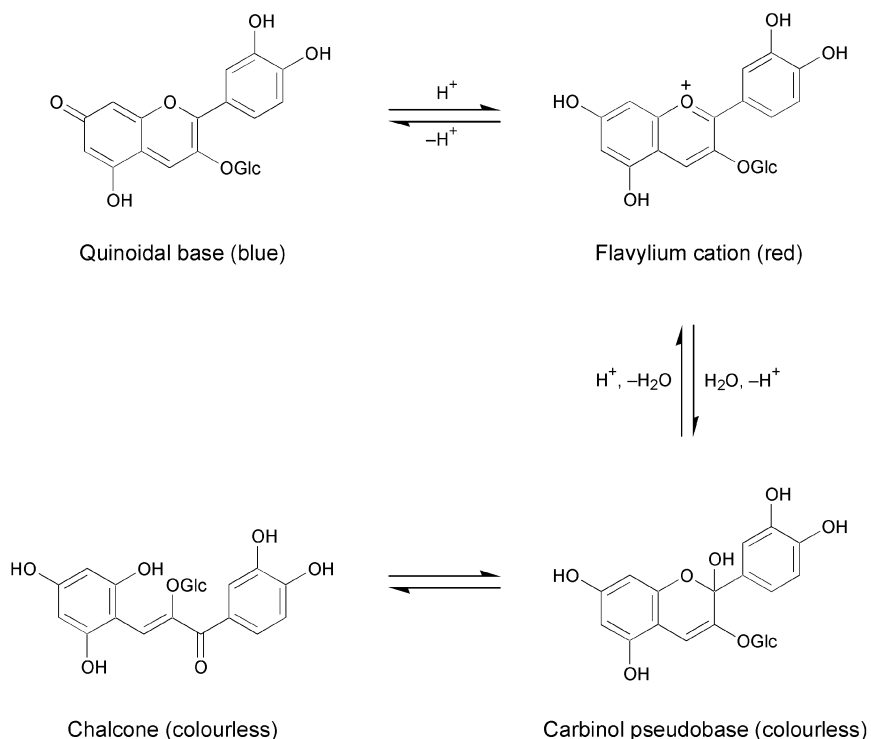
Illumination promotes isomerisation of carotenoids. During iodine-catalysed photoisomerisation of all-*trans*- $\alpha$ - and all-*trans*- $\beta$ -carotenes the major isomer was 13,15-di-*cis*-carotenes (Chen *et al.*, 1994). Different chlorophyll compounds may act as photosensitisers and when added to a solution of  $\beta$ -carotene, photoisomerisation may occur when exposed to light (O'Neil and Schwartz, 1995).

### 8.3.3 Flavonoids and betalains

#### *Anthocyanins*

These are inherently unstable and they are affected by pH, light, heat, oxygen, iron, copper, tin or ascorbic acid (Francis, 1985; Belitz and Grosch, 1987). Anthocyanins change colour in response to pH (Fig. 8.7). At a pH of approximately three or lower, the orange, red or purple flavylium cation exists. As the pH is raised, kinetic and thermodynamic competition occurs between the hydration reaction of the flavylium cation and the proton transfer reaction related to acidic hydroxyl groups. While the first reaction gives colourless carbinol pseudo-bases (Cheminat and Brouillard, 1986), the latter reactions give rise to more violet quinoidal bases. Although anthocyanins are unstable and easily destroyed during processing, this group of compounds are widely used as natural colorants in food (Adams, 1981).

The anthocyanins are in some cases influenced by co-pigmentation, which protects or restores stable and strongly absorbing forms of the anthocyanins. In



**Fig. 8.7** The effect of pH on interconversion and colour changes of anthocyanidins. Glc = glucose.

short, co-pigmentation involves stacking of the anthocyanin with molecules such as flavonoids, simple phenolic or aliphatic acids. Both inter- and intramolecular and self-association are involved in the co-pigmentation mechanisms (Goto, 1987; Mazza and Brouillard, 1987). Purple carrot, red radish and red cabbage rich in acylated anthocyanins, display great colour stability due to intramolecular co-pigmentation. Other vegetables rich in flavonols and with a high co-pigment/pigment ratio also have more stable colours (Malien-Aubert *et al.*, 2001).

### Betalains

The colour of betalains is less sensitive to pH changes than the colour of anthocyanins. The hue value of betalains is stable in solutions with pH between 3.5 and 7.0. However, addition of alkali destroys the red colour and causes degradation of the pigments (Watts *et al.*, 1993). Red and yellow betalain are thermolabile with and without the presence of oxygen and they are degraded by light (Drdak and Vallova, 1990). When beets are boiled, betalains leach into the cooking water. The colour fades, but usually the colour of betalains is so intense that the fading will not be noticed. Excessive heating, however, will turn the pigment brown (von Elbe *et al.*, 1974; Sapers and Hornstein, 1979; Francis,

2000). Metal cations, such as iron, copper, tin and aluminium, accelerate the degradation. Water activity also affects the betalains stability and/or the colour of products containing these pigments (von Elbe, 1987). In general, the greatest stability of betalains in foods or model systems is found with reduced exposure to light, oxygen and high humidity (von Elbe, 1977; Cohen and Saguy, 1983; Rayner, 1993).

## 8.4 Post-harvest influences on vegetable colour

The post-harvest period is the period from when a vegetable is harvested till it is consumed or further preserved. Vegetables are purchased whole or as washed, sorted, peeled, cut, sliced and otherwise partially processed. The latter method is called minimal processing and encompasses any short procedure that adds value (King and Bolin, 1989). Vegetative tissue is living, respiring and transpiring. During the post-harvest period, many chemical reactions are interacting due to the physiology of the plant tissue, microbial growth and the physical damage taking place during post-harvest handling and processing.

Temperature is the most influential factor on pigment degradation but, e.g., atmospheres and ethylene level can also have an effect. The rate of biological or biochemical reactions usually increases two- to threefold for every 10°C rise in temperature (Kader, 1987). For many vegetables, shelf-life can best be extended by lowering the storage temperature to slightly above the freezing point of the tissue (Price and Flores, 1993). Low storage temperatures reduce respiration and transpiration rates, inhibit enzymatic quality losses, slow down ethylene production and delay ripening and senescence. Modified (MA) or controlled atmosphere (CA) storage generally entails reduced oxygen and elevated carbon dioxide levels compared to normal air. These techniques have similar effects as lowering the temperature. CA is applied to vegetables in a continuous flow system. MA can be modified around the vegetable by build up of respiratory gases in semi-permeable plastic bags, pouches, or overwrapped trays or by injection of gases. Ethylene natural plant hormone found in all fruits and vegetables is a volatile compound produced in low concentrations by vegetables to promote and regulate many aspects of plant development. It is called the 'ripening hormone' because it accelerates post-harvest development and ripening, and it is involved in many aspects of growth regulation and senescence (Price and Flores, 1993). It is produced at increasing rates during maturation but also in response to physical injuries, disease incidence, increased temperatures up to 30°C and water stress (Kader, 1992; Bastrash *et al.*, 1993).

Vegetable tissues consist mainly of parenchyma cells. In intact cells many degradative enzymes are either inactive or separated from their respective substrate located in the vacuole. Minimal processing and other types of wounding disrupt the cells, allowing compartmented enzymes to be mixed with the substrates, thereby accelerating destruction and quality decline. When cells rupture, the plant will respond to this wounding. It implies activation of enzymes

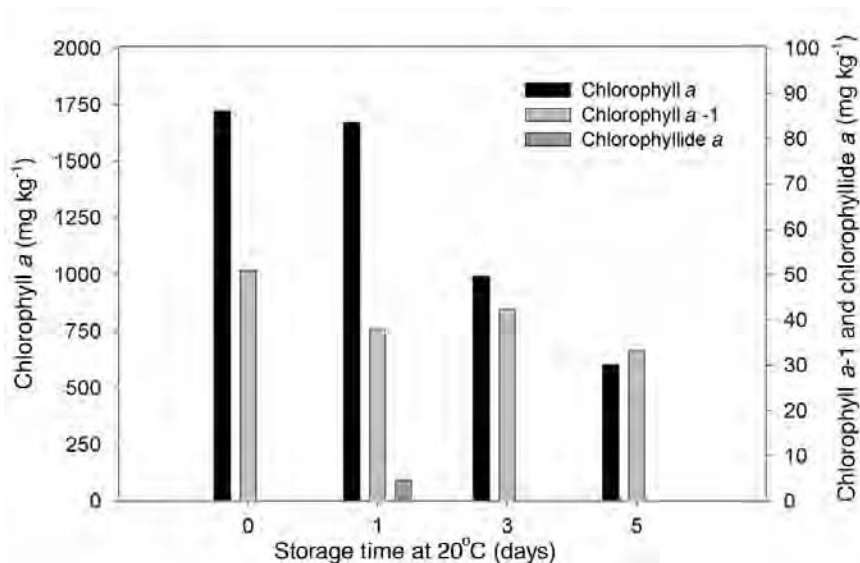
resulting in increased respiration, ethylene biosynthesis and other enzymatic changes (Price and Flores, 1993). Elevated respiration results in faster loss of nutrients, accelerates the onset of senescence, and limits shelf-life (Wills *et al.*, 1998).

#### 8.4.1 Chlorophylls

Loss of greenness is one of the major symptoms of post-harvest senescence in harvested green vegetables. In senescent leaves, the colour will change from green to yellow, red, and finally, brown due to breakdown of the chlorophylls into colourless or bleached products (Fig. 8.5), unmasking of the more stable carotenoids and, in some plants, to a *de novo* synthesis of anthocyanins (Gross, 1991). Senescence is best described as endogenously controlled deteriorative changes, which are natural causes of death in cells, tissues, organs or organisms (Noodén *et al.*, 1997). Environmental factors may trigger senescence or enhance it but do not regulate it. It is genetically regulated or controlled. Plant senescence is a multifactoral syndrome in which free radical metabolism seems to play an important role (Leshem, 1988).

Changes of colour in green vegetables often represent a loss of quality because the colour of a fresh or a processed product is expected to be as close as possible to the colour of the product at harvest. Gnanasekharan *et al.* (1992) registered surface colour changes in broccoli and cucumber stored up to 12 days at normal (broccoli 4°C; cucumber 10°C) and abuse temperatures (broccoli 21°C; cucumber 37°C). Hue angle, which describes the relationship between  $b^*/a^*$ , represented a reasonable prediction of sensory perceived change in colour in broccoli and cucumbers.

The rate of chlorophyll breakdown varies considerably from species to species. Philosoph-Hadas *et al.* (1994) studied natural senescence in detached leaves of edible watercress (*Rorippa nasturtium-aquaticum*), parsley (*Petroselinum crispum*) and sage (*Salvia officinalis*). Detached watercress leaves exhibited a very rapid senescence rate, about 60% of the initial chlorophyll content was lost within two days. The senescence rate of parsley was much slower showing no chlorophyll loss during the first day and a 70% loss after five days storage at 22°C. The senescence rate of detached sage leaves was much slower than those of the other two species. Chlorophyll degradation was paralleled by an increase in lipid oxidation. Plants with a high oxidative defence mechanism had a better chlorophyll retention than plants with a low defence mechanism in the presence of peroxide (Yamauchi *et al.*, 1980; Philosoph-Hadas *et al.*, 1994). Post-harvest loss of green colour or chlorophyll degradation has also been reported in cabbage (Heaton *et al.*, 1996b), green asparagus (Chang, 1987), broccoli (Zhuang *et al.*, 1994) and spinach (Yamauchi and Watada, 1991; Lopez-Ayerra *et al.*, 1998). In some commodities and under certain storage conditions, however, the tissues have a capacity to synthesise chlorophyll in the beginning of the post-harvest period followed by a later loss (Zhuang *et al.*, 1994).



**Fig. 8.8** Changes in the chlorophyll *a* and its derivatives during five days storage of parsley leaves at 20°C. Data from Yamauchi and Watada (1993).

Yamauchi and Watada (1993) studied natural senescence of parsley leaves. When chlorophyll *a* decreased, chlorophyll *a*-1 increased to about 3% of the content of chlorophyll *a* (Fig. 8.8). With storage, a small accumulation of chlorophyllide *a* was noted, but the accumulation did not continue nor was it retained and it did not increase to the extent of the amount of chlorophyll *a* lost. The pheophytin *a* content was in general low and no pheophorbides were detected leaving the formation of colourless by-products as the only explanation of chlorophyll loss during post-harvest senescence (Heaton and Marangoni, 1996a).

Ethylene hastens the rate of chlorophyll degradation in vegetables whereas temperature and controlled atmosphere (CA) retards it. Lers *et al.* (1998), showed that ethylene accelerated the initial chlorophyll degradation in parsley leaves stored eight days in the dark at 25°C. In contrast, elevated CO<sub>2</sub> retarded all senescence processes and high CO<sub>2</sub> nullified the senescence inducing effect of ethylene. Paradis *et al.* (1996), showed that the chlorophyll content in broccoli was maintained throughout six weeks storage in 2% O<sub>2</sub> + 6% CO<sub>2</sub> at 4°C. In contrast, 60% of the chlorophyll was lost during four weeks storage in air. When the florets were transferred to air after CA storage, however, the chlorophyll content decreased and the flower buds yellowed and opened rapidly. In conclusion, green chlorophylls convert to colourless products during natural senescence unmasking the yellow and orange carotenoids during post-harvest storage.

### 8.4.2 Carotenoids

As described in Section 8.4.1, the degradation of the chlorophylls results in yellow coloration of senescing green leaves. Degradation of the carotenoids also occurs during senescence, but with a slower rate than the chlorophylls. The carotenoid composition may also change during senescence, causing formation of xanthophyll esters and epoxides (Biswal, 1995).

Temperature and storage conditions may affect the degradation and/or the carotenogenesis of the carotenoids, depending on the species (Takama and Saito, 1974; Lee, 1986). In corn and sweet pepper, elevated storage temperatures resulted in higher losses of carotenoids as compared to storage under optimum temperature conditions (Quackenbush, 1963; Takama and Saito, 1974; Gross, 1991). In sweet pepper, 20% of the total carotenoid content was lost during nine days at 7°C, whereas 60 and 80% was lost during the same period at 15°C and 17°C, respectively (Takama and Saito, 1974). Synthesis of carotenoids has been reported in roots and fruit vegetables during post-harvest storage, the rate being dependent on commodity and storage temperature (Lee, 1986; Gross, 1991; Minguez-Mosquera *et al.*, 1994; Rodriguez-Amaya, 1997). Lee (1986) found that the content of several carotenes, including  $\alpha$ - and  $\beta$ -carotene, increased slightly in carrots during 100 to 125 days of storage at 2°C and then decreased. The optimum temperature for rapid development of the red colour in tomatoes was reported to be between 20 to 25°C. Below 10°C and above 30°C the colour development was inhibited. After seven days at 35°C the tomatoes turned yellow, probably as a consequence of inhibition of the lycopene biosynthesis above 30°C, whereas the biosynthesis of  $\beta$ -carotene continued. Carotenogenesis also depend on the ripeness of the fruit at harvest (Inaba *et al.*, 1996; Gross, 1991).

The presence of light during post-harvest storage may also influence carotenoid stability, dependent on the commodity. In carrots, the all-*trans*- $\beta$ -carotene,  $\alpha$ -carotene and lutein content were very stable during eight days storage at 4°C in both light and dark. However, the losses of violaxanthin and lutein in spinach were 60% and 22%, respectively, during eight days of storage in light (Kopas-Lane and Warthesen, 1995).

Literature is scarce on the effects of controlled atmosphere (CA) and modified atmosphere (MA) storage on carotenoid content and colour retention in yellow and red vegetables. Sozzi *et al.* (1999), reported a lower content in total carotenoids and lycopene in tomatoes, which had been stored in 3% O<sub>2</sub> or 20% CO<sub>2</sub> than after storage in air (control). No differences were found after storage in low O<sub>2</sub> and high CO<sub>2</sub>. When the samples were transferred to air, the total carotenoid and lycopene contents were lower in the tomatoes, which were subjected to different storage treatments from those stored in air all the time.

### 8.4.3 Flavonoids and betalains

The effect of post-harvest storage on the flavonoid content in spinach has been studied (Gil *et al.*, 1999). The total flavonoid content (approximately 1000 mg



kg<sup>-1</sup> fresh weight) was almost constant during three and seven days storage at 10°C in air and MA. The stability of anthocyanins in red cabbage was studied during 84 days storage at 4°C (Lachman *et al.*, 1991). During this period, both colour and the anthocyanin content were maintained in two red cabbage cultivars. The reason for this superior stability were due to glucosylation of cyanidin and acylation with several aromatic acids. The dominant structures in red cabbage are cyanidin 3,5-diglucoside and cyanidin 3-sophoroside-5-glucoside acylated with sinapic acid, ferulic acid, *p*-coumaric acid, or caffeic acid (Idaka *et al.*, 1987; Ikeda *et al.*, 1987; Nakatani *et al.*, 1987). Highly acylated anthocyanins have also been found in purple sweet potatoes (Otake *et al.*, 1992; Goda *et al.*, 1997; Rodriguez-Saona *et al.*, 1998; Lewis *et al.*, 1998; Terahara *et al.*, 1999). The colour stability of petanin isolated from purple potatoes was studied at different pH values (Fossen *et al.*, 1998). After 60 days storage at 10°C and pH 4.0 more than 84% of the anthocyanin pigment was intact.

The effect of cold storage on the content of betalains was determined in the peel of red beet (Kujala *et al.*, 2000). The concentration of betanin ranged from 15.500 to 38.700 mg kg<sup>-1</sup> fresh weight. The amounts of betanin decreased during 140 days storage at 5°C after which the level began to rise during the next 46 days. The increase of betanin content after 140 days could be rationalised by the ability of red beet pigments to degrade and regenerate continuously during storage, as the reaction is reversible (Han *et al.*, 1998). Osornio and Chaves (1998) studied the effects of temperature and atmospheric composition on the content of betalains and surface colour (hue angle) in grated beet. The beets were washed, grated, packed in trays and wrapped with polyvinylchloride (PVC) or ethylene-vinyl acetate (EVA) films, respectively. The trays were stored at 0°C or 4°C for seven days and then transferred to 20°C for one day. In general, the content of betalains decreased during seven days storage. At 0°C, the betalains content decreased by 40–50% after seven days, whereas at 4°C the decrease was greater. The PVC film gave a better retention of betalains as compared to the EVA film. Hue angle and chroma, the objective colour measurements, were affected by film permeability, storage temperature and time.

## 8.5 Heating and vegetable colour

Several industrial processes associated with heating may have an impact on colour. Vegetables are blanched, cooked, boiled, canned, pasteurised or dehydrated. Vegetables are also brined or fermented using processes that may cause colour changes.

- Blanching (70–105°C) is a short heat treatment in order to inactivate enzymes and stabilise the vegetables against deterioration during prolonged storage. Optimum blanching condition for vegetables is individual, depending on the type and size of the plant tissue and thus the time and temperature necessary to inactivate particular enzymes (Williams *et al.*, 1986). Peroxidase is one of

the most heat stable enzymes in vegetables and it is often used as an index of blanching. Blanching can be conducted in water, steam or by using microwave energy (Williams *et al.*, 1986). Blanching is often used prior to freezing.

- Cooking or boiling are often used during home preparation or re-heating.
- Retorting by canning is generally conducted at 121°C for 25–40 minutes or at 115°C for 30–40 minutes. The primary objective of canning is to produce sterile vegetables. The target criterion is to destroy spores of *Clostridium botulinum*, the most heat-resistant food-poisoning bacteria (Shin and Bhowmik, 1995).
- Pasteurisation (60–85°C) is associated with destruction of vegetative micro-organisms (Williams *et al.*, 1986). Pasteurisation of vegetable products is primarily used in juice processing.
- Dehydration includes removal of water from the solids to a level at which microbial spoilage is minimised, which makes the vegetables stable during long-term storage. Water can be removed by freeze drying, spray drying or cabinet drying. Dehydrated vegetables are used in food mixtures, dry salad dressing mixes, dehydrated soups and convenience foods (Luning *et al.*, 1995).
- Brining and fermentation include preservation by the use of acids. Acids are added in the brining process. In fermentation, however, the process can be self-driven (natural) or acids can be added to drive the process towards optimal conditions.

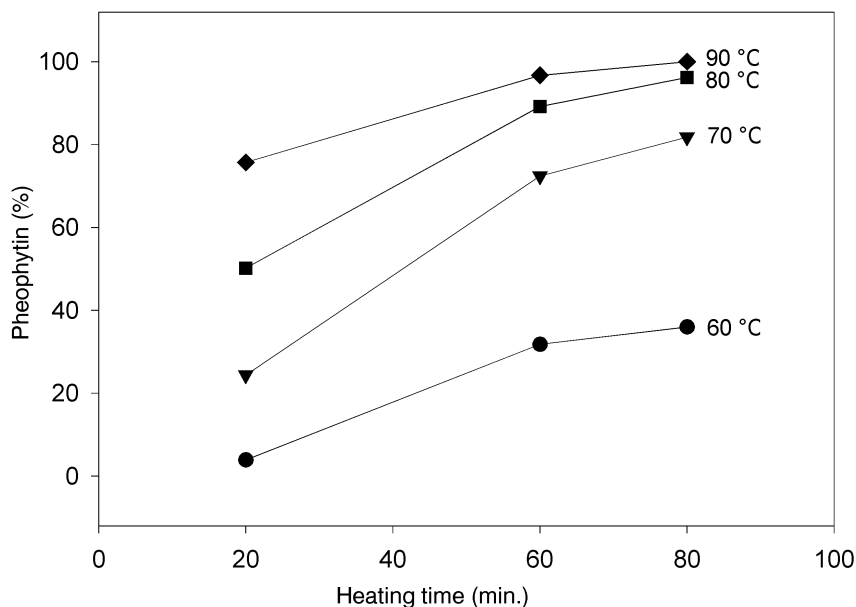
### 8.5.1 Chlorophylls

Maintaining the colour during thermal processing of green vegetables is still an industrial challenge. There is a strong consumer preference for heated green vegetables with clear bright green colours (Haisman and Clarke, 1975). Heating is necessary to stabilise the vegetables and to inactivate quality-deteriorating enzymes. At temperatures above 60°C, the living cell is killed, pectic substances are broken down and the cell structure is irreversibly changed (Katsaboukakis, 1983). In fermentation, where heat is not used, however, microbial growth may cause a similar breakdown of the cellular structure (Gunawan and Barringer, 2000). Chlorophyll is located in the grana of the chloroplast in the intact cell. When the cell is killed during heating, the chloroplast envelope disappears, the grana disperse and globules of lipid material sheathed in vestigial membranes are the only remnants of the chloroplast structure that are left (Haisman and Clarke, 1975). When the cytoplasmic membranes disrupt, the membrane permeability increases and cellular acids are released, leading to degradation of chlorophylls to, for example, pheophytins, a process that cannot take place until the membrane array of the chloroplast has been disorganised (Haisman and Clarke, 1975).

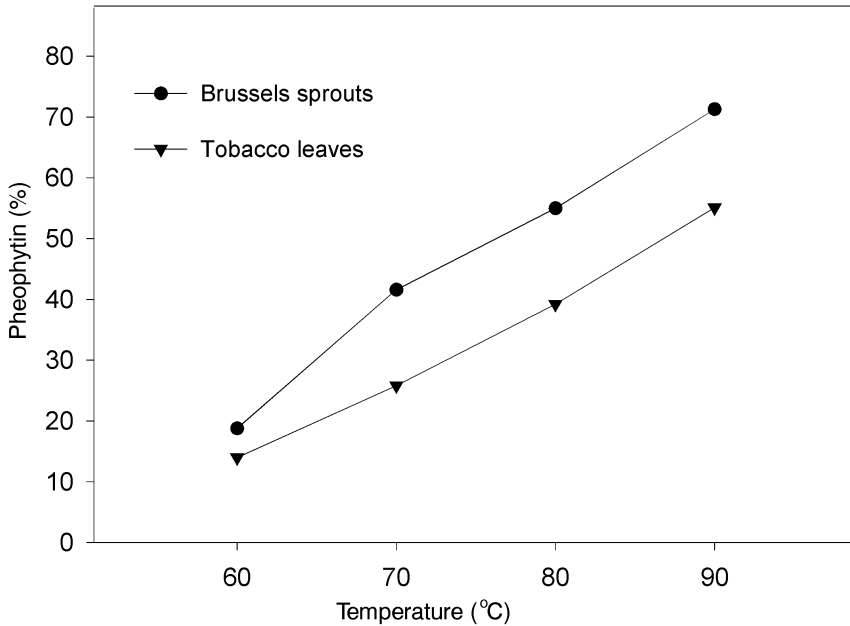
Walker (1964) proposed that the ratio of chlorophyll to pheophytin could be used as a colour index for heated green vegetables. Reflectance measurements of

greenness ( $-a^*$ ) or its combinations ( $-a^*/b^*$  or  $-La^*/b^*$ ) have also been used for objective evaluation of the total colour change during heating of green vegetable purée and juice (Hayakawa and Timbers, 1977; Shin and Bhowmik, 1995; Steet and Tong, 1996; Weemaes *et al.*, 1999). In green asparagus the hue angle was selected to represent the colour changes of the spear surface during heating (Lau *et al.*, 2000).

Conversion of chlorophyll to pheophytin during heating depends on temperature, the length of the heat treatment and pH (Ryan-Stoneham and Tong, 2000). At 60°C and above, chlorophyll is progressively converted to pheophytin, the rate increases rapidly as the temperature rises (Fig. 8.9). Several studies reveal that pheophytinisation and loss of green colour follow a first-order reaction mechanism (Shin and Bhowmik, 1995; Steet and Tong, 1996; Gunawan and Barringer, 2000; Lau *et al.*, 2000; Ryan-Stoneham and Tong, 2000). Chlorophyll *a* is more heat sensitive than chlorophyll *b*. In broccoli juice, chlorophyll *a* degraded twice as fast as chlorophyll *b* (Weemaes *et al.*, 1999). A two-step degradation of chlorophyll and change in green colour were observed in heated broccoli juice especially at 90 and 100°C. The first step was pheophytinisation and the second step decomposition of pheophytins to pyropheophytins (Fig. 8.5). This phenomenon was attributed to the molecular environment around the chlorophyll in the broccoli juice. Haisman and Clark (1975) demonstrated that 55% of the chlorophylls was converted to pheophytin during 20 min. heating of Brussels sprouts at 80°C. In contrast, only 39% was converted in tobacco leaves (Fig. 8.10). The pH of the Brussels sprouts and the



**Fig. 8.9** Changes in the relative content of pheophytin in sugar beet leaves after 20, 60 and 80 minutes heating at 60, 70, 80 and 90°C. Data from Haisman and Clarke (1975).



**Fig 8.10** Changes in relative content of pheophytin in Brussels sprouts and tobacco leaves after 20 minutes heating at 60, 70, 80 and 90°C. Data from Haisman and Clarke (1975).

tobacco leaves after heating was 6.2 and 6.0, respectively. Haisman and Clarke (1975) examined the rate of pheophytin formation at 60°C in 25 plant tissues with pH ranging from 4.1 to 7.2. They observed a general increase in pheophytin with decreasing pH, however, the overall correlation between pheophytin and pH was poor. In green beans immersed in water at 60°C, the pheophytin conversion was 12 times faster than in the leaves from the same plant although both tissues had virtually the same pH (Haisman and Clarke, 1975). Haisman and Clark (1975) concluded that chlorophyll stability in damaged cells depends on the concentration and nature of the cellular acids and the association between the chlorophyll and the lipoproteins in the chloroplast.

### Blanching

Underblanching of vegetables may cause colour changes during frozen storage due to chlorophyllase and peroxidase activity (Williams *et al.*, 1986). In secondary reactions, lipid hydroperoxides and hydroperoxy radicals produced by lipoxygenase may cause bleaching of the chlorophylls and loss of colour. Overblanching may result in an undesirable colour due to complete pheophytinisation, formation of pyropheophytin and breakdown of chloroplasts (Chen and Chen, 1993). Using water blanching, water will eventually enter the cell and the intercellular spaces, chloroplasts swell and chlorophylls diffuse through the cell and into the blanching media (Katsaboxakis, 1983). Ponne *et al.*

(1994) studied the effect of hot water, steam and microwave-steam blanching on the colour quality of endive and spinach. The products were blanched until no peroxidase activity could be observed. The microwave-steam blanched vegetables scored highest on general acceptability by the panellists although water blanched endive and spinach resembled the fresh product most in colour. The effect of hot-water, steam and microwave blanching showed that there was no consistent difference in colour between the treatments (Drake *et al.*, 1981; Stone and Young, 1985).

### *Cooking*

Three minutes cooking of green peas (*Pisum sativum*) in water had no significant effect on the total chlorophyll content, but the chlorophyll *a* and *b* contents decreased and the chlorophyll *a'* and *b'* and pheophytin *a* and *b* contents increased, respectively (Edelenbos *et al.*, 2001). Chen and Chen (1993) reported changes in the chlorophyll-related compounds during microwave heating of sweet potato leaves (Table 8.4). During eight minutes cooking, the total chlorophyll content decreased approximately seven-fold. Chlorophyll *a* was gradually converted to chlorophyll *a'*, a chlorophyll *a* isomer and pheophytin *a*, while chlorophyll *b* was converted to chlorophyll *b'* during the first two minutes. Pheophytin *b* and pyropheophytin *a* were observed after eight minutes cooking.

### *Canning and pasteurisation*

In canned and pasteurised green vegetables the colour will eventually change from blue-green (chlorophyll) to olive-green (pheophytin) due to the severe and long heat treatments required to achieve commercial sterility. López-Ayerra *et al.* (1998) found that all the chlorophyll in spinach was converted to pheophytins during 30 minutes retortment at 121°C. In contrast, only 16% of the chlorophyll was lost during frozen storage. Conventionally canned, green vegetables primarily contain olive-brown pheophytin *a* and *b* and pyropheophytin *a* and *b* (Schwartz and Lorenzo, 1991; Gauthier-Jaques *et al.*, 2001).

**Table 8.4** Changes in the chlorophyll content (mg kg<sup>-1</sup>) during microwave cooking of sweet potato leaves

Pigment group	Heating time, minutes			
	0	2	4	8
Chlorophyll <i>a</i> + <i>b</i>	1152	528	359	49
Chlorophyll <i>a'</i> + <i>b'</i>	89	237	149	15
Chlorophyll <i>a</i> + <i>b</i> isomers	nd <sup>1</sup>	20	14	57
Pheophytin <i>a</i> + <i>b</i>	nd	17	36	45
Pyropheophytin <i>a</i> + <i>b</i>	nd	nd	nd	17
Total chlorophylls	1241	802	558	183

<sup>1</sup> Not detected. From Chen and Chen (1993).

### Dehydration

Long dehydration times, especially at high temperatures, lead to poor-quality products due to caramelisation, Maillard reactions, enzymatic reactions and pigment degradation such as pheophytinisation (Horner, 1993). Pheophytinisation occurs at a very low water potential also during storage (Lajolo and Lanfer-Marquez, 1982). In parsley leaves dried at temperatures between 80 and 90°C, 3–7 % of the chlorophylls were converted to pheophytins as compared to 12–15% in samples dried at temperatures between 100 and 140°C (Berset and Caniaux, 1983). When the dried samples were stored for two years, however, samples dried at a lower temperature contained a higher percentage of pheophytins than samples dried at a higher temperature. During long-term storage, chlorophyll *b* is more stable than chlorophyll *a*. This may affect green colour perception, as chlorophyll *b* is yellow-green and chlorophyll *a* is blue-green (Forni *et al.*, 1988). In bell peppers, the loss of green colour was higher at 70 and 75°C than at 55 and 60°C, respectively (Sigge *et al.*, 1999). In general, the loss of chlorophyll was minimised during cabinet drying at 30°C. Rocha *et al.* (1993) compared the chlorophyll loss in steam-blanching and non-steam-blanching leaves of basil (Table 8.5). In general, the chlorophyll loss was minimised after drying when steam blanching was compared to no blanching prior to drying. The steam treatment increased the cell wall permeability, favoured water migration and reduced the drying times.

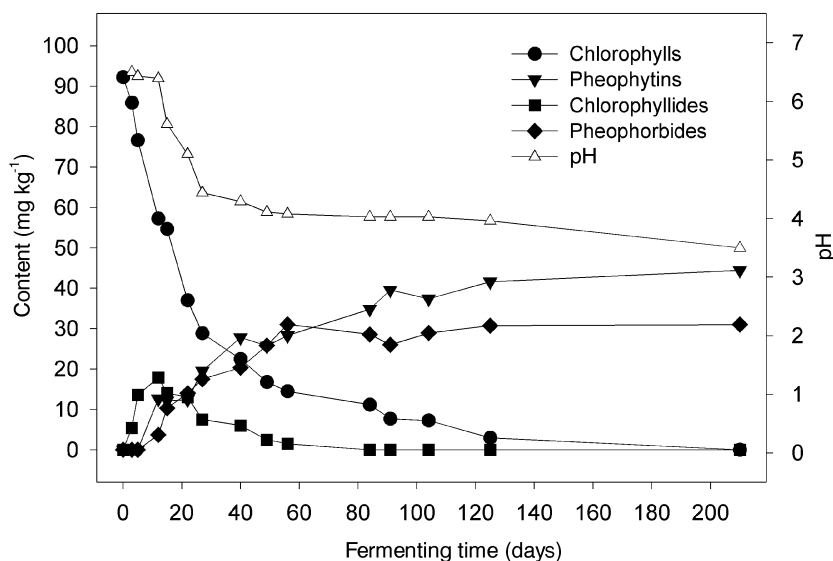
### Brining and fermentation

Acid preservation or usage of a vinegar-based salad dressing will affect green colour retention in green vegetables. This was first observed during brining of cucumbers (Gross, 1991), but later also during fermentation of olives and green cabbage (Minguez-Mosquera *et al.*, 1989; Heaton and Marangoni, 1996). In coleslaw processing, cabbage is finely shredded and packed under mild pressure in airtight containers. An acid dressing might be added before air is excluded. The added acid drastically reduces the pH of the product,

**Table 8.5** The effect of steam blanching and drying temperature on chlorophyll retention in basil leaves

Treatment	Drying temperature	Chlorophyll <i>a</i> + <i>b</i>	Pheophytin <i>a</i> + <i>b</i>
Fresh		100	100
Steam blanching	40°C	99	158
	60°C	69	134
No blanching	40°C	60	81
	60°C	37	161

From Rocha *et al.* (1993).



**Fig. 8.11** Changes in the chlorophylls and pH during olive fermentation. Data from Minguez-Mosquera *et al.* (1989).

creating conditions favourable for fermentation, but also for chlorophyll degradation. In olive processing where pH changes slowly, the major degradation pathway is to chlorophyllides and further to pheophorbides (Fig. 8.11). The conversions of chlorophyll to chlorophyllide and pheophytin to pheophorbide are a result of chlorophyllase activity while degradation of chlorophyll to pheophytin and chlorophyllide to pheophorbide is a result of acidic pH (Heaton *et al.*, 1996a). Apparently, salad dressings containing simple, hydrophilic acids such as citric, malic and acetic acids give a better colour retention in green vegetables than dressings containing acids with a benzene ring (e.g., benzoic acid) (Gunawan and Barringer, 2000). Acids with a hydrophobic group like the benzene ring seem to be able to diffuse through the lipid membrane of the chromoplast and dissociate the hydrogen ion inside the cell in contrast to the hydrophilic acids.

### 8.5.2 Carotenoids

In general, the effect of thermal heating on degradation and isomerisation of carotenoids in yellow, orange and red vegetables depends on the vegetable species, the carotenoid composition and the heating method and processing conditions. It is well known that time, temperature and the presence of oxygen are factors that affect carotenoid stability (Khachik *et al.*, 1992; van der Berg *et al.*, 2000).

### *Blanching and cooking*

Blanching of carrots leads to a brighter colour and a higher degree of colour saturation than no heating (Mirza and Morton, 1977). The heating process results in chromoplast disintegration, carotenes being dissolved in the cellular lipids, leading to a colour shift (Purcell *et al.*, 1969). The effect of heating on the carotenoid content is complex. The carotenoid content may increase, decrease or be maintained during thermal processing (Park, 1987; Chandler and Schwartz, 1988; Gross, 1991; Khachik *et al.*, 1992; Lessin *et al.*, 1997). A mild heat treatment at temperatures below 100°C for a short time, e.g., blanching, may increase the carotenoid content per unit weight. This increase is a consequence of enhanced chemical extractability due to cell wall disruption, loss of moisture and soluble solids and inactivation of the carotene oxidising enzymes (Ogunlesi and Lee, 1979; Park, 1987; Chandler and Schwartz, 1988; Lessin *et al.*, 1997; Granado *et al.*, 1992; Oruña-Concha *et al.*, 1997; Howard *et al.*, 1999; van der Berg *et al.*, 2000). However, a severe heat treatment may result in degradation of carotenoids leading to a decrease in the carotenoid content. During heating, isomerisation may occur, depending on the length and severity of the heat treatment (Kläui and Bauernfeind, 1981; Park, 1987; Chandler and Schwartz, 1988; Khachik *et al.*, 1992; Lessin *et al.*, 1997).

In sweet potato strips, Chandler and Schwartz (1988) found a 13 and 4% increase, respectively, in the total  $\beta$ -carotene content after two and ten minutes blanching in water at 100°C. However, after 80 minutes baking at 191°C a loss of 31% was reported. During heating, *cis*- $\beta$ -carotene isomers were formed accounting for 8, 15 and 23%, respectively, after 2, 10 and 80 minutes heating. In green peas, Edelenbos *et al.* (2001) also reported increases in the lutein content (38%),  $\beta$ -carotene content (23%) and total carotenoid content (26%) during three minutes boiling in water. In tomato, the lycopene content did not change during 4, 8 and 16 minutes cooking at 100°C (Thompson *et al.*, 2000). These results were in agreement with Khachik *et al.* (1992), who found that the lycopene content in tomatoes was stable during heating. Microwave heating for seven minutes at 6000 W of sweet potatoes resulted in a 23% loss of total  $\beta$ -carotene (Chandler and Schwartz, 1988). Microwave heating for nine minutes, however, had a minimal effect on the  $\beta$ -carotene content in carrots compared to no heating (Howard *et al.*, 1999). Barrett *et al.* (2000) measured the hue angle of unblanched and water blanched sweetcorn on the cob. The lowest value, indicating a yellow colour, was observed in unblanched samples increasing to higher values (more yellow-orange colour) with increasing blanching times.

Differences in the colour and the carotenoid content in relation to heating depend on the processing method (Glasscock *et al.*, 1982; Drake and Carmichael, 1986; Park, 1987). In general, carotenoids will not leach into the surroundings during water blanching or cooking due to their hydrophobic nature. No significant differences were detected in the colour of carrots that were blanched in water for two minutes at 88°C or using High Temperature Short Time (HTST) blanching at 45 and 60 psi for 30 to 60 seconds, respectively (Drake and Carmichael, 1986). Others (Glasscock *et al.*, 1982; Bognár, 1987;



Park, 1987) also reported no significant differences in the  $\beta$ -carotene content or colour between conventional and microwave cooked carrots, showing that the methods and the processing conditions had similar effects. However, different commodities or different processing conditions may result in inconsistent results. Drake *et al.* (1981) found that water-blanching sweetcorn was superior in yellow colour to microwave blanched corn.

The presence of oxygen may result in carotenoid degradation during blanching. Blanching carrot slices pretreated with nitrogen sparge, blanching and frozen with oxygen sparge before storage at  $-40^{\circ}\text{C}$  for four weeks, showed the most pronounced degradation in total carotenoids and the lowest hue angle value (brighter colour) than freezing with nitrogen sparge (Talcott and Howard, 1999). Reduced oxygen concentration during processing will prevent carotene bleaching significantly.

### *Canning*

Retorting temperatures (e.g.,  $115^{\circ}\text{C}$  and  $121^{\circ}\text{C}$ ) destroy individual carotenoids in carrot juice, canned carrots and sweet potatoes (Ogunlesi and Lee, 1979; Chandler and Schwartz, 1988; Chen *et al.*, 1995; Howard *et al.*, 1999). Retorting with increasing temperatures substantially increases the *cis*-isomerisation and reduces the carotene content because of enhanced oxidation and isomerisation (Ogunlesi and Lee, 1979; Chandler and Schwartz, 1988; Lessin *et al.*, 1997; van der Berg *et al.*, 2000). Ogunlesi and Lee (1979) reported an increase in the *cis* isomers of  $\alpha$ - and  $\beta$ -carotene after retortment of carrots and subsequently a 35% decrease in all-*trans*- $\beta$ -carotene and a 26% decrease in all-*trans*- $\alpha$ -carotene. The retorting conditions were  $115^{\circ}\text{C}$  for 30 minutes. Lessin *et al.* (1997) found an increase of 10 to 39% in the content of *cis*-isomers in ten canned vegetable products. A lower total content of all-*trans*-carotenoids and higher total content of *cis*-isomers were obtained in sweet potatoes retorted at  $121^{\circ}\text{C}$  for 23 minutes than at  $116^{\circ}\text{C}$  for 34 minutes (Lee, 1974). Subsequent oxidation of the carotenoids can be prevented by exclusion of oxygen during the canning process (Desobry *et al.*, 1998).

### *Juice processing*

Carrot and tomato juice is produced from red and yellow vegetables. The colour and stability of the carotenoids in these products depends on the processing conditions. In carrots, the carotenoids are more exposed to oxidation because the vegetables are macerated prior to the heat treatment (van der Berg *et al.*, 2000). The processing conditions also have a significant effect on the carotenoid retention. Chen *et al.* (1995) compared canning of carrot juice at  $121^{\circ}\text{C}$  for 30 minutes with acidification, acidification plus heating at  $105^{\circ}\text{C}$  for 25 seconds, high temperature short time treatment (HTST) at  $110^{\circ}\text{C}$  for 30 seconds and HTST at  $120^{\circ}\text{C}$  for 30 seconds. The various treatments resulted in carrot juices with colours from orange (acidification, acidification plus heating at  $105^{\circ}\text{C}$  for 25 seconds) to yellow (canning) with the highest destruction of the carotenoids obtained by canning (Chen *et al.*, 1995). Munsch *et al.* (1983) demonstrated that

the colour of processed carrot juice correlated well with the carotenoid content and the formation of *cis* isomers. The colour intensity was reduced as *cis* isomers were formed (Bauernfeind, 1981). In tomato juice, 1% of the lycopene was lost during seven minutes heating at 90°C as compared to 17% during seven minutes at 130°C (Miki and Akatsu, 1970)

### *Dehydration*

In general, dehydration results in severe degradation and isomerisation of the carotenoids, especially if the dried vegetables are not kept unprotected from light and air in an inert N<sub>2</sub> or CO<sub>2</sub> atmosphere or under vacuum (Lovric *et al.*, 1970; Gee, 1979; Villota *et al.*, 1980; Kläui and Bauernfeind, 1981; Goldman *et al.*, 1983). Discolouration of dried vegetables is promoted by high-temperature drying (Chandler and Schwartz, 1988; Shi *et al.*, 1999; Zanoni *et al.*, 1999; Krokida *et al.*, 2001). Chemical treatments, e.g., addition of 0.05% (w/v) sulphite and 2.5% (w/v) corn starch resulted in a reduced carotene loss and a better colour quality of carrot dices, which were dehydrated in air at 70°C for four hours or 90°C for two hours (Zhao and Chang, 1995). In tomatoes, Zanoni *et al.* (1999) reported a 10% loss in the lycopene content in tomato during drying at 110°C, however, at 80°C the lycopene content was unchanged. Shi *et al.* (1999) studied the lycopene content and the colour of tomatoes after dehydration in air at 95°C for 6–10 hours, vacuum-drying at 55°C for 4–8 hours, or osmotic treatment at 25°C in 65 Brix sucrose solution for 4 hours, followed by vacuum drying at 55°C for 4–8 hours. The highest lycopene loss (4%) was observed in air-dried samples containing approximately 17% *cis*-isomers, showing that isomerisation and oxidation occurred simultaneously. Osmotic-vacuum dried samples resulted in the lowest lycopene loss (2%) followed by vacuum drying (3%). In these samples, *cis*-isomers accounted for 7% and 10%, respectively. These differences were probably due to the sugar remaining on the surface during osmosis, preventing oxygen from penetrating and oxidising the lycopene. The colour quality, expressed as the  $a^*/b^*$  ratio, was significantly lower in the air-dried than in the other samples (Shi *et al.*, 1999). Drum drying of sweet potato flakes at 160°C and 25 rpm resulted in a 21% loss in carotene (Chandler and Schwartz, 1988). No significant changes in the total carotenoid content and the colour were reported in freeze-dried carrots although freeze-drying usually results in products with a higher porosity that may favour oxidation of carotenoids (Desobry *et al.*, 1998; Krokida *et al.*, 2001). However, freeze-drying takes place under vacuum and therefore oxygen is almost excluded protecting the carotenoids from degradation. Tang and Chen (2000) found that the degradation of carotenoids in dried carrot pulp depends on the subsequent storage temperature and illumination time. The content of all-*trans*- $\alpha$ -carotene, all-*trans*- $\beta$ -carotene and all-*trans*-lutein decreased with increasing storage temperature (4°C, 25°C or 45°C) and illumination time while the *cis*-isomerisation increased. Storage of tomato powder in N<sub>2</sub> or air resulted in a lycopene retention of 53% and 8%, respectively (Lovric *et al.*, 1970).

### 8.5.3 Flavonoids and betalains

Various transformations of anthocyanins occur during heating producing yellowish or brownish pigments (Duhard *et al.*, 1997). During processing, the equilibrium shifts towards the colourless carbinol base and chalcone forms (Fig. 8.7). However, the original anthocyanin colour usually is regained during cooling (Bridle and Timberlake, 1997). Physical damage such as peeling, cutting and slicing disrupts the membranes and allows mixing of enzymes and substrates previously in separate compartments. The enzymes are inactivated by the heat process, however, elevated temperatures shift the anthocyanin equilibrium towards the chalcone form. Differences in the stability between anthocyanins are probably related to their degree of acylation. Acylated from cabbage, for example, anthocyanins are more stable than the non-acylated anthocyanins (Davidek *et al.*, 1990). Highly acylated compounds also have another feature that makes them desirable. They usually have an extra absorption band at 560–600 nm and/or at 600–640 nm in weak acid or neutral solutions (Shi *et al.*, 1992). This means that the compounds are highly coloured at pH above 4.0, where non-acylated anthocyanins are nearly colourless. The two extra bands result from a tendency of anthocyanins to form quinoidal base structures in weakly acid or neutral solutions (Fig. 8.7). The colour of red cabbage extracted and buffered to pH 3.0, 3.5 and 4.0 and heated at 80°C showed that the anthocyanins were stable (Sapers *et al.*, 1981; Duhard *et al.*, 1997).

Heat degradation of betanin is a first-order reaction. The decomposition of betanin at 60°C and 80°C showed that the reaction rate was doubled by reducing the pH from 6.2 to 3.3 (Patkai and Barta, 1996). Patkai and Barta (1996) reported the loss of colour during beetroot processing at various temperatures and pH, using UV measurements at 533 nm. Heat degradation of betanin has also been reported by Saguy (1979), Cohen and Saguy (1983) and Havlikova *et al.* (1985).

## 8.6 Freezing and vegetable colour

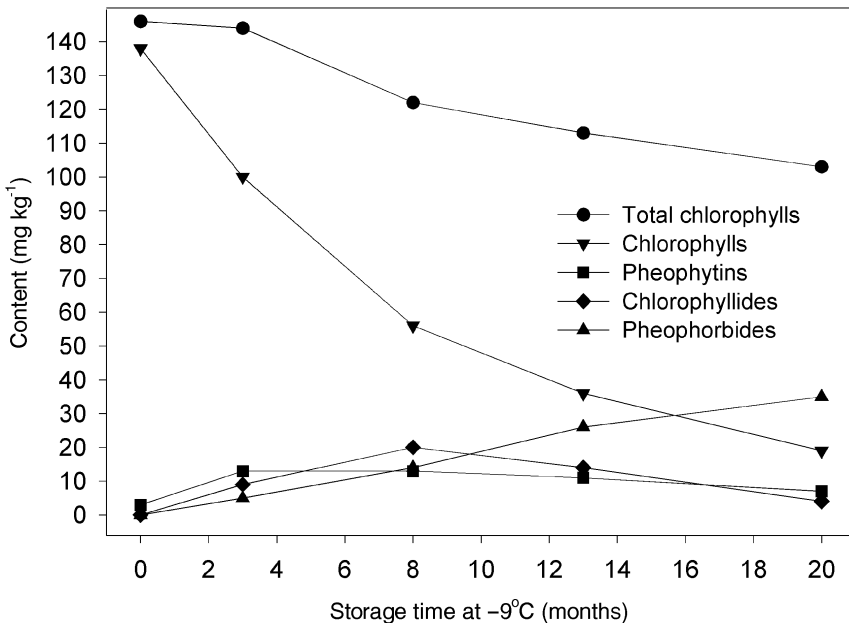
Freezing is one of the most important methods for retaining the quality of vegetables during long-term storage. Most vegetables are blanched prior to freezing. However, some vegetables, e.g., leek and carrots may be frozen raw. The quality of frozen vegetables delivered to the consumer can be no better than their quality at the time of freezing. Storage temperatures, storage time and temperature fluctuations in the store are the principal factors affecting the quality of frozen vegetables. The storage life of nearly all frozen vegetables increases with colder storage temperatures to at least between –25 and –40°C (Cano, 1996).

### 8.6.1 Chlorophylls

Green peas, green beans, Brussels sprouts and spinach are the most consumed frozen green vegetables. These vegetables are blanched prior to freezing to

prevent enzymatic discolouration and off-flavour development. Pigment degradation is related to storage temperature, the kind of processed vegetable, the necessary preparation steps of the crop prior to blanching and blanching and freezing time (Dietrich *et al.*, 1959). Blanched green beans lost 10% of the initial chlorophyll content within 0.7 and 10 months at  $-7$  and  $-18^{\circ}\text{C}$ , respectively. In blanched leaf spinach and green peas, 10% of the initial chlorophyll content was lost within 1.6 and 2.5 months storage at  $-7^{\circ}\text{C}$ , respectively. At  $-18^{\circ}\text{C}$ , 10% of the chlorophyll content was lost within 30 and 43 months, respectively (Dietrich *et al.*, 1959). During prolonged storage of frozen green vegetables the colour changes as a consequence of chlorophylls being converted to the corresponding pheophytins (Fig. 8.5). However, chlorophyll bleaching is also reported (Walker, 1964).

Buckle and Edwards (1970) followed the chlorophyll degradation in unblanched green peas stored at  $-9^{\circ}\text{C}$  in  $\text{N}_2$  during 20 months (Fig. 8.12). The chlorophyll content decreased and the content of chlorophyllide, pheophytin and pheophorbide increased. However, the increase could not explain the total loss. It is chlorophyllase and to a lesser extent peroxidase that are primarily responsible for the pigment conversion in unblanched and minimal blanched frozen green vegetables (Williams *et al.*, 1986). Lipid hydroperoxides and hydroperoxy radicals produced by lipoxygenase are involved in secondary reactions causing chlorophyll bleaching. Recently, Oruña-Concha *et al.* (1997) studied the effects of 12 months frozen storage at  $-22^{\circ}\text{C}$  on the chlorophyll *a*



**Fig. 8.12** Interconversion of chlorophylls in unblanched peas during storage in nitrogen for 20 months at  $-9^{\circ}\text{C}$ . Data from Buckle and Edwards (1970).

and *b* content in unblanched and blanched green beans and peppers. In unblanched beans, the chlorophyll content decreased during the first months and then stabilised. Similar results were obtained for blanched beans, but here the decrease was due to blanching. A convincing mechanism of chlorophyll degradation during frozen storage of green vegetables is still not proposed because the methods used previously to identify and quantify chlorophyll degradation products have been prone to error. Recently, Gauthier-Jaques *et al.* (2001) published results on an improved HPLC method to track more than 30 chlorophyll related compounds from processed spinach. This method could track all coloured pigments, however, fluorescent detection is needed to track all the colourless chlorophyll derivatives (Heaton and Marangoni, 1996).

### 8.6.2 Carotenoids

The carotenoids of frozen yellow, orange and red vegetables are relatively stable. For this reason, the colour is also rather stable. No or minor degradation or isomerisation of carotenoids have been reported in frozen carrots, sweet potatoes and corn (Park, 1987; Chandler and Schwartz, 1988; Gross, 1991; Rodrigues-Amaya, 1997; Kidmose and Martens, 1999; van der Berg *et al.*, 2000). Freezing and six months frozen storage of sweet potatoes at  $-17^{\circ}\text{C}$  did not result in any changes in the total or isomerised content of carotenes nor in the colour of the product (Chandler and Schwartz, 1988; Collins *et al.*, 1995). However, oxygen and light may affect carotenoid stability during frozen storage. Therefore optimal packaging of the vegetables before frozen storage can exclude light and oxygen and thus give a better carotenoid and colour retention (Oruña-Concha *et al.*, 1997). In general, blanching of yellow, orange and red vegetables prior to freezing preserves the carotenoids. The carotenoids resist degradation during frozen storage better blanched than unblanched (Oruña-Concha *et al.*, 1997; van der Berg *et al.*, 2000). One reason may be the inactivation of lipoxygenase. In blanched carrots,  $\beta$ -carotene decreased slightly during 12 months frozen storage at  $-20^{\circ}\text{C}$ , whereas a significant decrease was observed in raw frozen carrots, the decrease being related to cultivar (Crivelli and Polesello, 1973; Howard *et al.*, 1999). In tomato pulp, however, a continuous decrease of the carotenoid content and in the red colour was observed during a period of 24 months at  $-20^{\circ}\text{C}$  and  $-25^{\circ}\text{C}$ , respectively (Urbányi and Horti, 1989).

Few studies deal with the effect of different freezing methods on carotenoid content (Drake *et al.*, 1981; Kidmose and Martens, 1999). Drake *et al.* (1981) found that sweetcorn that were individually quick-frozen at  $-23^{\circ}\text{C}$  with a belt speed at 10 min/rev., had a better yellow colour than blast-frozen samples at  $-23^{\circ}\text{C}$ . Kidmose and Martens (1999) did not find a significant difference in the carotene content of blast- and cryogenic-frozen carrot slices. In contrast, Sulc (1973) reported that freezing of red pepper in liquid nitrogen at  $-30^{\circ}\text{C}$  gave a better red colour retention than freezing by conventional methods. Long thawing may be detrimental to the carotenoid retention. Thawing of unblanched, frozen

carrots for six hours at 22°C resulted in a 12% loss of the initial carotene content (Park, 1987)

### 8.6.3 Flavonoids and betalains

To our knowledge, no data are available on flavonoids and betalains in relation to frozen storage.

## 8.7 Maintaining vegetable colour

Any change in colour during post-harvest storage or processing represents a loss of quality as compared to the colour at harvest. Therefore the aim of all treatments will be to prevent colour changes.

### 8.7.1 Chlorophylls

Maintaining green colour in vegetables during post-harvest storage and industrial processing is still a technological challenge. In the post-harvest industry, all treatments that retard the natural senescence processes, e.g., the conversion of chlorophylls into colourless degradation products will improve the retention of green colour. In the processing industry, all treatments that hinder conversion of chlorophyll to pheophytin, pyropheophytin, pheophorbide or pyropheophorbide will maintain the clear bright green colour of the vegetables.

#### *Post-harvest*

Several techniques may be used to maintain green colour after harvest, e.g., storage under optimal temperature conditions, exclusion of ethylene or light, CA or MA storage, hot water treatment or changes in the genetic manifestation of the plant material. Temperature is the most important single factor in retarding natural senescence in harvested vegetables (Kader, 1992) therefore, any deviation from the optimum temperature will reduce the storage life of green vegetables. In addition to optimum temperature storage, CA and MA storage has been successfully applied to further slow down natural senescence in green vegetables. In green beans, for example, the storage life was prolonged three days under 1% O<sub>2</sub> + 3% CO<sub>2</sub> as compared to air storage due to a better retention of the green pigments and pod colour (Cano *et al.*, 1998). Pretreatment with hot water at 47°C for 7.5 minutes also prolonged the storage life of broccoli florets (Tian *et al.*, 1996). The major problem with all post-harvest techniques is, however, to find the optimum storage conditions. Vegetables have diverse morphological structures, composition, and different physiology (Kader, 1992).

In general, ethylene is a very damaging plant hormone to all green vegetables as it accelerates natural senescence even in trace amounts (< 0.1 ppm). For this reason, it is important to keep green vegetables separated from fruits, which are characterised by a moderate to high ethylene production rate (1–100 ppm). The

negative effects of ethylene can be minimised by excessive venting or removal by oxidation with potassium permanganate. The use of 'stay-green' or persistent-green mutants has been suggested to overcome the negative effects of natural senescence in green beans (Dean, 1968). 'Stay-green' plants are characterised by having tissues that hinder or have reduced chlorophyll degradation capacity even when dried.

In potatoes, greening is a significant problem during retail exposure. At the ultrastructural level, greening of potatoes involves transformation of amyloplasts into chloroplasts and once formed the chloroplasts are stable and the green colour persistent (Gross, 1991). The simplest way to prevent chlorophyll formation in potatoes is to store them in the dark, or alternatively, to use light-absorbing films or illuminate with light at 750 nm (Adams, 1996).

### *Heating*

In general, any mild heat treatment prior to freezing or dehydration will improve colour retention in green vegetables. Several other methods have been suggested to improve green colour retention during blanching, sterilisation or dehydration. Seow and Lee (1997) found that four hours infiltration with calcium under vacuum (pH 5.6 and 25°C) prior to water blanching (100°C for four minutes) slightly improved the green colour of beans and bell peppers by strengthening the tissues against chlorophyll degradation and heat damage. The calcium pretreatment had no detectable effect on the taste of the vegetables. In contrast, addition of  $\text{CaCl}_2$  to the blanching medium decreased chlorophyll stability in peas blanched for three minutes at 90°C (Selman, 1987). In the latter experiment, calcium infiltration and blanching occurred simultaneously. Therefore, the positive effect on the cell walls could not overcome the negative effect of the divalent cations on pH (LaBorde and von Elbe, 1994b).

High-temperature, short-time treatment (HTST) is another method for preserving the green colour of vegetables. Schwartz and Lorenzo (1991) found that continuous aseptic processing of spinach purée using two minutes pre-blanching of the leaves at 57°C, puréeing, brining, high-temperature, short-time processing (5.3 seconds at 142°C) and packaging could preserve the initial chlorophyll to some extent, but eventually the chlorophyll was lost during storage. Metal complexes of chlorophyll, where metals such as zinc and copper are introduced into the chlorophyll porphyrin ring, have long been known to be more resistant to acid and heat and thus to prevent loss of green colour (von Elbe *et al.*, 1986). Formation of green metal complexes during thermal processing and subsequent storage is known as 'regreening'. As an example, the Veri-Green process is a patented procedure by which blanching of green vegetables is performed in the presence of zinc (II) salts. The regreening of Veri-Green processed beans has mainly been attributed to the formation of zinc complexes of pheophytin and pyropheophytin (Segner *et al.*, 1984; von Elbe *et al.*, 1986; Canjura *et al.*, 1999; Gauthier-Jaques *et al.*, 2001). The formation of complexes depends on pH, the zinc (II) and the pigment concentration (LaBorde and von Elbe, 1990 and 1994a). Many countries have, however, restricted the zinc (II)

ion concentration in the final product. The colour of dehydrated green vegetables may be improved using freeze-drying or methods that increase the drying rate during dehydration. In freeze-dried, rehydrated spinach, chlorophyll *a* (green) and pheophorbide *a* (brown) were the most prominent products of the chlorophyll (Gauthier-Jaques *et al.*, 2001). In conventional dehydration, a combination of osmotic dehydration (40% sucrose + 20% citrate at 30°C for 24 hours) with hot-air drying at 65°C seemed to minimise the detrimental effects of heat on colour retention in green peas (Ertekin and Cakaloz, 1996). Osmotic dehydration reduced the initial water content by 70–75%, shortened the air-drying time, improved the rehydration characteristics and these factors had a positive effect on colour retention (Ertekin and Cakaloz, 1996).

### 8.7.2 Carotenoids

In general, treatments that exclude oxygen, light and elevated temperatures will reduce the degradation of carotenoids. In addition, natural antioxidants, e.g., phenolics, may inhibit the negative effects of lipoxygenases, resulting in reduced oxidation and bleaching (Fig. 8.6) (Oszmianski and Lee, 1990; Gross, 1991). The inhibitory effect of phenolics varies with the chemical structure of the compounds and concentration. Flavans have a stronger inhibitory effect on lipoxygenase than flavonols followed by phenolic acids, e.g., ferulic acid and *p*-coumaric acid (Oszmianski and Lee, 1990). The inhibitory effect on carotene bleaching increases with increasing phenolic concentration. Broccoli phenolics showed the strongest inhibition on carotene bleaching followed by phenolics from peas, carrots, beans and spinach (Oszmianski and Lee, 1990).

A 5% solution of grounded tomato seeds and 0.2% dry rosemary leaves reduced carotenoid degradation during frozen storage, especially when the tomato pulp was exposed to light. The ground tomato seeds accelerated the colour loss during the first six weeks of frozen storage. However, after ten weeks storage it stabilised the colour compared to the untreated tomato samples (Biacs and Wissgott, 1997). Addition of tomato to a recipe enhances the stability of  $\beta$ -carotene during processing and storage probably because of the antioxidative and the protective effects of lycopene (Rodrigues-Amaya, 1997).

### 8.7.3 Flavonoids, anthocyanins and betalains

Low-temperature and CA storage are the most promising techniques to prevent post-harvest losses of flavonoids, anthocyanins and betalains (King and Bolin, 1989). No information is given in the literature on processing and colour loss caused by convention of flavonoids, anthocyanins and betalains. However, in the food colour industry, the colour of red cabbage extracts was stabilised by adding water-soluble antioxidants and phosphates to the extracts (Washino and Moriwaki, 1990). Furthermore, addition of flavonol glycosides enhanced the co-pigmentation of the extracts (Nishimura *et al.*, 1990; Washino and Moriwaki, 1990).



## 8.8 Future trends

During the last decade, there has been increasing interest in using more gentle and differentiated processing methods to minimise the changes in colour, texture, taste and nutritional quality of vegetables after harvest. Production of convenience food by minimal processing methods is an area of rapid development. Aseptic processing using high-temperature short-time (HTST) heating is a method that seems to have the potential in liquid and puréed foods to replace retortment or pasteurisation. Many efforts have been made over the years to optimise the blanching process towards efficient and gentle blanching. Short blanching times may result in a higher sensory and nutritional quality, have an impact on colour and pigment stability and reduce energy and water costs. The major problem with all gentle processing methods is, however, to find the optimum processing conditions as these are a function of morphological structure, composition, and general physiology of the vegetable raw material. Often very sophisticated and highly developed process techniques are needed to control and adapt the process to optimum conditions. The use of various additives may be an alternative to flexible, differentiated and gentle processing methods. Many efforts are made to prevent discoloration of vegetables after harvest including CA or MA and/or various chemical treatments. Pre-treatment of green vegetables with calcium prior to blanching to reduce the loss of chlorophyll has been suggested. Replacement of magnesium with zinc or copper has been used in the processing of Veri-green beans to stabilise the chlorophyll structure and the colour of green vegetables. The loss of carotenoids due to oxidation during dehydration has been prevented by the use of starch coating. In the western world, there will be an increasing demand for high-quality processed vegetable products with a colour and pigment composition as close to 'natural' as possible and with other sensory quality attributes intact.

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## Modelling colour stability in meat

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### 9.1 Introduction

The colour stability of meat products is influenced by a large number of factors, some being of a biochemical nature and some due to handling during the slaughter process. In addition to these factors are packaging and storage conditions. This chapter focuses on modelling the effect of the external factors applied during packaging and storage. However, meat from different sources shows different tendencies to undergo colour deterioration and this variation in internal factors influences the developed models therefore some consideration will also be given to discussing how internal factors, e.g., muscle type and addition of nitrite in cured meat, affect the models. Modelling can be used to identify the most important factors/interaction of factors affecting quality loss and to define critical levels of these factors, thereby forming the basis for proposing the optimal packaging and storage conditions or the best compromise if several deteriorative reactions need to be considered. Caution in choosing the optimal packaging and storage conditions can largely improve the colour and shelf life of meat products.

When packaging fresh meat products an elevated oxygen ( $O_2$ ) partial pressure needs to be maintained to keep the meat pigment myoglobin in its oxygenated bright red state. Through modelling of a MAP system for fresh beef, the most critical external factors have been identified to be storage temperature and gas composition (Jakobsen and Bertelsen, 2000). Through modelling of a MAP system for cured meat products, the most critical external factors have been identified to be a low availability of oxygen combined with exclusion of light (storage temperature was kept constant at 5°C) (Møller *et al.*, 2002). However, low availability of oxygen is not solely ensured by reducing the



residual oxygen level in the headspace during the packaging process. Other equally critical factors are a high product-to-headspace ratio and a packaging film of low oxygen transmission rate (OTR) of the packaging film (Møller *et al.*, 2002).

## 9.2 External factors affecting colour stability during packaging and storage

Modified atmosphere packed meat is a complex and dynamic system where several factors interact (Zhao *et al.*, 1994). Models can be used to describe how the initial package atmosphere changes over time and how these changes affect product quality and shelf life. The dynamic changes in headspace gas composition during storage can be modelled as a function of gas transmission rates of the packaging material, initial gas composition, product and package geometry, gas absorption in the meat, etc. Combined with the knowledge from models on quality changes in the meat as a function of packaging and storage conditions such as storage temperature, gas composition and light exposure, predictions of product shelf life can be made. Pfeiffer *et al.* (1999) developed simulations of how product shelf life changes with different packaging and storage conditions for a wide range of food products (primarily dry products). However, at present sufficient models for many quality deteriorative reactions are lacking and only few attempts have been made to model chemical quality changes in meat products, in contrast to modelling of microbial shelf life, where extensive work has been performed (McDonald and Sun, 1999).

## 9.3 Modelling dynamic changes in headspace gas composition

### 9.3.1 Permeability of the packaging film

Headspace gas composition changes dynamically due to several factors. Gas exchange with the environment occurs over the packaging film if the partial pressure of a gas differs on the two sides of the film. The amount of gas that permeates the film can be calculated from equation 9.1 (Robertson, 1993):

$$Q = P \cdot \Delta p \cdot t \cdot A \quad 9.1$$

where

$Q$  = the amount of gas that permeates the film ( $\text{cm}^3$ )

$P$  = the permeability of the packaging film ( $\text{cm}^3/\text{m}^2/24\text{h}/\text{atm}$ )

$\Delta p$  = the difference in gas partial pressure on the two sides of the film (atm)

$t$  = the storage time (24h)

$A$  = the area of the package ( $\text{m}^2$ )

Different gases have different permeability through the same film. For conventional films, the permeability of  $\text{CO}_2$  is generally 4–6 times larger than

that of O<sub>2</sub> and 12–18 times greater than that of N<sub>2</sub>. The permeability of a plastic film is roughly proportional to the thickness of the film. Doubling the film thickness approximately halves the permeability of the film.

Permeability is also influenced by storage temperature and relative humidity. Pfeiffer *et al.* (1999) found that the empirical equation 9.2 fitted well with published data for oxygen permeability.

$$P(T, RH) = \exp (c_0 + c_1/T + c_2 \cdot RH + c_3 \cdot RH^2) \quad 9.2$$

where

$P$  = the permeability of the packaging film

$T$  = storage temperature

$RH$  = storage relative humidity

$c$ 's = an experimental derived coefficients.

Gas exchange over the packaging film is of particular importance when the film needs to maintain a narrowly defined gas concentration, as shown in the example in Section 9.5, where the permeability of even small amounts of O<sub>2</sub> into a package containing a cured meat product is considered a critical packaging parameter.

### 9.3.2 Gas absorption in the meat

Headspace gas composition can also change due to gas absorption in the meat. Packaging in elevated levels of CO<sub>2</sub> can result in large amounts of CO<sub>2</sub> being absorbed in the meat (Jakobsen and Bertelsen, 2002; Zhao *et al.*, 1994) changing drastically the gas composition initially applied. Absorption of O<sub>2</sub> and N<sub>2</sub> is negligible compared to the absorption of CO<sub>2</sub> (Jakobsen and Bertelsen, 2002). Models for CO<sub>2</sub> solubility as a function of packaging and storage parameters such as product to headspace volume ratio, temperature and initial CO<sub>2</sub> level were developed by Zhao *et al.* (1995) and Devlieghere *et al.* (1998). Fava and Piergiovanni (1992) developed models of CO<sub>2</sub> solubility as a function of different compositional parameters,  $a_w$ , pH, protein, fat and moisture content. As regards gas absorption, equilibrium is obtained during the first one or two days. Microbial or meat metabolism can also cause slight changes in gas composition by using O<sub>2</sub> and producing CO<sub>2</sub>.

When it is understood how the gas atmosphere can change under different packaging and storage conditions from the initially applied atmosphere, this knowledge can be used to evaluate the effect on deteriorating quality reactions. Besides microbial growth, the primary concern when packaging both fresh and cured meat products is colour stability. The mechanisms of colour changes in fresh meat products and cured meat products are completely different as can be seen from the examples on modelling given in the following two sections.

## 9.4 Modelling in practice: fresh beef

Jakobsen and Bertelsen (2000) and Bro and Jakobsen (2002) modelled colour stability of fresh beef under different packaging and storage conditions. In all cases colour measurements were performed using a Minolta Colorimeter CR-300 (Minolta, Osaka, Japan) using the  $L^*$ ,  $a^*$ ,  $b^*$  coordinates (CIELAB colour system). Red colour was expressed as the  $a^*$ -value, the higher the  $a^*$ -value the redder the sample. When packaging fresh red meats elevated  $O_2$  partial pressures are used to stabilise myoglobin in its bright red oxygenated form (oxymyoglobin). However, elevated  $O_2$  levels may increase some deteriorative reactions, e.g., lipid oxidation. Consequently, it is interesting to investigate if a level of  $O_2$  exists, which is acceptable when considering both colour stability and lipid oxidation. Jakobsen and Bertelsen (2000) investigated different packaging and storage conditions (Table 9.1) and developed a regression model/response surface model predicting the colour  $a^*$ -value as a function of storage time, storage temperature and  $O_2$  level based on steaks of *Longissimus dorsi* muscles from four different animals. The resulting model (equation 9.3) contains main effects of the three factors plus two-way interactions and two squared effects. Interpretation of the model is best done by exploring the response surface plot (Fig. 9.1).

$$a^*\text{-value} = \beta_0 + \beta_1 \cdot \text{Day} + \beta_2 \cdot \text{Temp} + \beta_3 \cdot O_2 + \beta_4 \cdot \text{Day} \cdot \text{Temp} + \beta_5 \cdot \text{Day} \cdot O_2 + \beta_6 \cdot \text{Temp} \cdot O_2 + \beta_7 \cdot \text{Day} \cdot \text{Day} + \beta_8 \cdot \text{Temp} \cdot \text{Temp} \quad 9.3$$

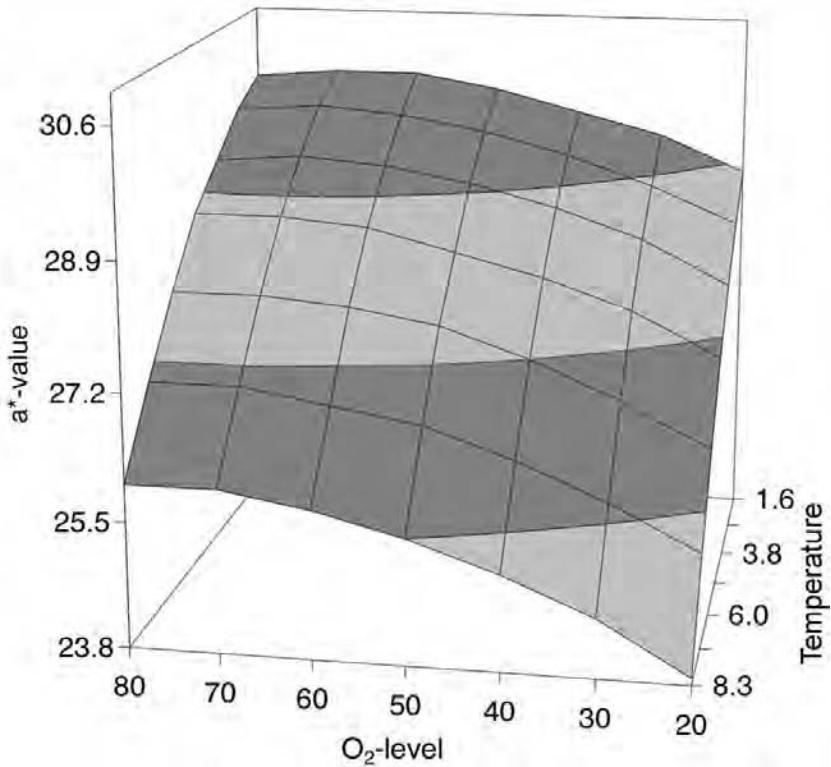
where the  $\beta$ s are regression coefficients.

Figure 9.1 shows a response surface plot varying the two factors, temperature and  $O_2$  level, while keeping the third factor, storage time, constant at day no. 6. Figure 9.1 reveals an interval of approximately 55–80%  $O_2$ , where the  $O_2$  level does not affect the colour  $a^*$ -value significantly (the nearly horizontal lines in this interval means that only temperature influences the  $a^*$ -value). The borders of this interval change a little depending on the setting of the day. But it is evident that the  $O_2$  level can be reduced from the normally used 70–80% without an adverse effect on the colour shelf life.

The complexity of the interactions/squared terms in eqn 9.3 called for further search for adequate models. A novel approach called GEMANOVA (Generalized Multiplicative ANOVA) was therefore used in Bro and Jakobsen

**Table 9.1** Packaging and storage conditions used in the models developed in Jakobsen and Bertelsen (2000)

Modelling factor	Abbreviation	No. of levels	Setting of levels
Storage time (days)	Day	5	2, 4, 6, 8, 10
Temperature ( $^{\circ}\text{C}$ )	Temp	3	2, 5, 8
$O_2$ level (%)	$O_2$	5	20, 35, 50, 65, 80



**Fig. 9.1** Response surface plot of predicted  $a^*$ -values (average of four animals) after six days storage at different temperatures and different oxygen levels (adapted from Jakobsen and Bertelsen, 2000).

(2002). In this study the effect of different packaging and storage conditions (Table 9.2) on colour stability and lipid oxidation of steaks of *Longissimus dorsi* muscles from three different animals was investigated. The effect of light was evaluated as the time of exposure to a fluorescent tube commonly used for retail display (Philips Fluotone TLD 18w/830 yielding 1000 lux at the package surface for 0, 50 or 100% of the storage time). Even when considering only two factor interactions a traditional ANOVA model for the experiment in Table 9.2 would look like equation 9.4 (before removal of any insignificant effects).

$$\begin{aligned}
 a^*\text{-value} = & \beta_0 + \beta_1 \cdot \text{Day} + \beta_2 \cdot \text{Temp} + \beta_3 \cdot \text{Light} + \beta_4 \cdot \text{O}_2 + \\
 & \beta_5 \cdot \text{Day} \cdot \text{Temp} + \beta_6 \cdot \text{Day} \cdot \text{Light} + \beta_7 \cdot \text{Day} \cdot \text{O}_2 + \\
 & \beta_8 \cdot \text{Temp} \cdot \text{Light} + \beta_9 \cdot \text{Temp} \cdot \text{O}_2 + \beta_{10} \cdot \text{Light} \cdot \text{O}_2 \quad 9.4
 \end{aligned}$$

where  $\beta$ s are regression coefficients.

On the contrary, when applying the GEMANOVA model the interactions are modelled as one higher-order multiplicative effect, resulting in the eqn 9.5

**Table 9.2** Packaging and storage conditions used in the models developed in Bro and Jakobsen (2002)

Modelling factor	Abbreviation	No. of levels	Setting of levels
Storage time (days)	Day	4	3, 7, 8, 10
Temperature (°C)	Temp	3	2, 5, 8
Light exposure (%)	Light	3	0, 50, 100
O <sub>2</sub> level (%)	O <sub>2</sub>	3	40, 60, 80

(before removal of any insignificant effects). The interpretation of the GEMANOVA model is much more simple than the ANOVA model as is discussed in detail in (Bro, 1997) and (Bro and Jakobsen, 2002).

$$a^*\text{-value} = \text{Day} \cdot \text{Temp} \cdot \text{Light} \cdot \text{O}_2 \quad 9.5$$

The resulting GEMANOVA model for the data in Table 9.2 can be written as equation 9.6, since the effect of the O<sub>2</sub>-level is insignificant in the interval between 40–80% O<sub>2</sub> (Bro and Jakobsen 2002). The interaction term Day · Temp · Light · cO<sub>2</sub> describes deviations from the a\*-value on day 0 in a very simple way, and interpretation of the model parameters can be performed from Fig. 9.2.

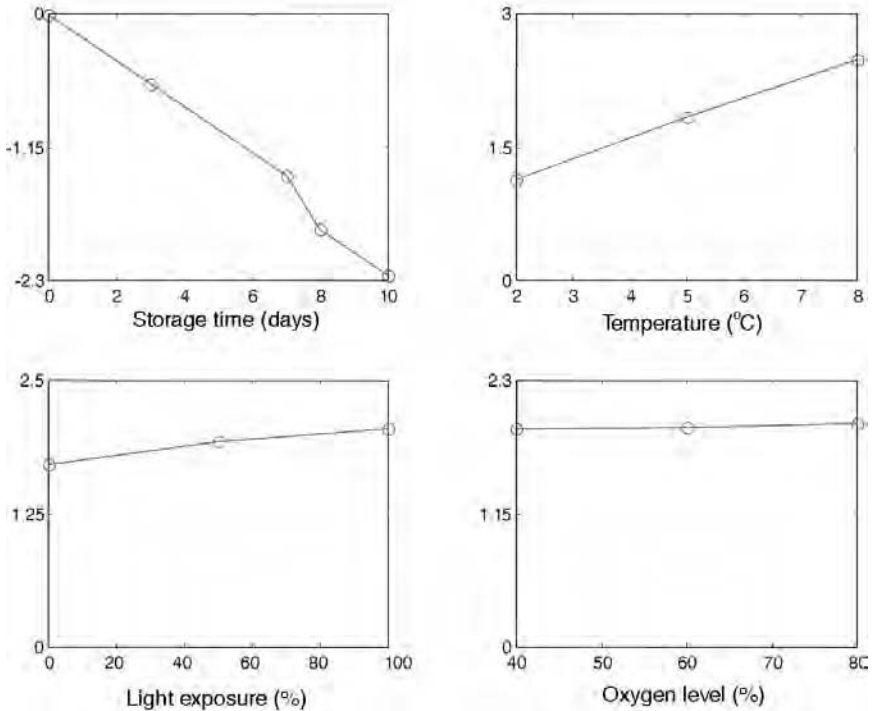
$$a^*\text{-value} = a^*\text{-value}_0 + \text{Day} \cdot \text{Temp} \cdot \text{Light} \cdot \text{cO}_2 \quad 9.6$$

where a\*-value<sub>0</sub> is the a\*-value at day no. 0 and cO<sub>2</sub> is a constant.

For all settings of the factors the estimated response is simply the starting level of the a\*-value plus the product of the four effects seen from the ordinates in Figure 9.2. The multiplicative term in eqn 9.6 is 0 on day 0. Furthermore it is easily seen that:

- All changes in colour a\*-value are negative (colour becomes less red) compared to the starting colour. The change is calculated as the product of the four parameters Day, Temp, Light and O<sub>2</sub>, which consist of one negative number (Day) and three positive numbers.
- The changes are relative and the effect of the individual factors can be interpreted individually. For example, when going from 2°C to 8°C the temperature loading increases from 1.2 to 2.4 meaning that, regardless of all other factors, the decrease in a\*-value at 8°C is twice the decrease at 2°C.
- The effects of storage time and temperature are most important.
- The effect of light is minor although an increase in time of exposure to light seems to result in a decreased colour a\*-value.
- The effect of the O<sub>2</sub> level is insignificant in the interval from 40–80% and is therefore contained in eqn 9.6 as a constant.

The GEMANOVA model confirms the results from Jakobsen and Bertelsen (2000) by emphasising the importance of keeping a low storage temperature and showing no effect of O<sub>2</sub> level in the interval between approximately 40–80%. However, the interpretation of the model is much more simple, since the effect



**Fig. 9.2** Parameter levels for the interaction term Day · Temp · Light · CO<sub>2</sub> in equation 9.6 (adapted from Bro and Jakobsen, 2002).

of each factor can be interpreted individually. Likewise, applying the GEMANOVA model on the data set in Table 9.1 results in eqn 9.7 which is much more simple to interpret than eqn 9.3.

$$a^*\text{-value} = a^*\text{-value}_0^+ + \text{Day} \cdot \text{Temp} \cdot \text{O}_2 \quad 9.7$$

where  $a^*\text{-value}_0$  is the  $a^*\text{-value}$  at day no. 0.

From Fig. 9.3 the effect of the individual factors can be interpreted, and the stable interval between 40–80% O<sub>2</sub> is evident.

It is rather surprising, that 40% O<sub>2</sub> is sufficient to ensure the stability of the bright red meat colour, as an O<sub>2</sub> level of 70–80% is commonly used in the industry. The applied product to headspace volume ratio for the experiments in Tables 9.1 and 9.2 was approximately 1:9. The large headspace volume might cause only minor changes in headspace gas composition (oxygen partial pressure) to take place during storage. However, when packaging fresh meat products for retail sale, a large headspace volume is common. Furthermore, large amounts of oxygen have to permeate over the film or be used for meat/microbial metabolism before a noteworthy change in oxygen partial pressure will take place and the meat colour will be affected. A reduction in the applied oxygen level leaves the possibility of using more carbon dioxide or nitrogen in the package headspace.

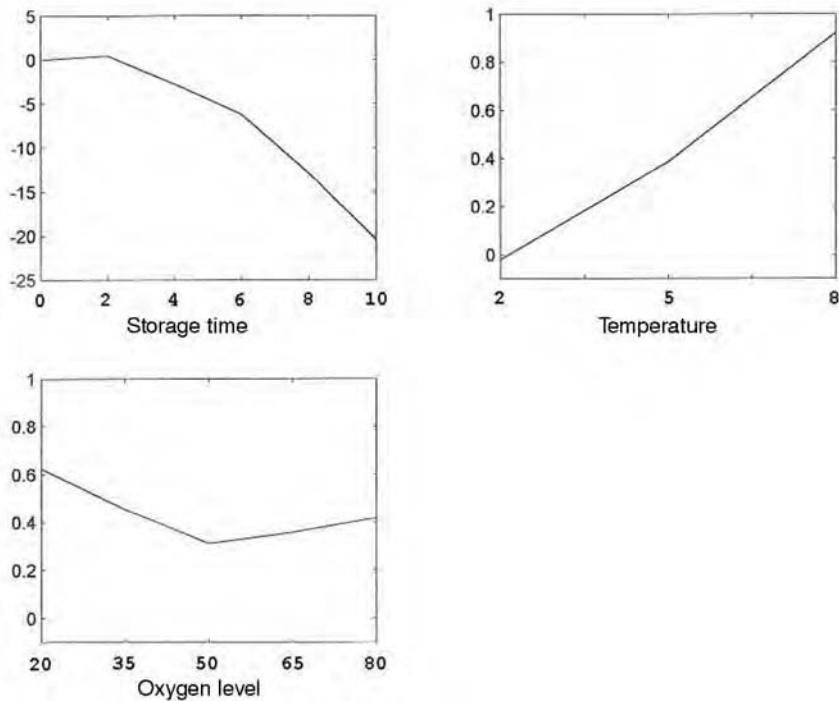


Fig. 9.3 Parameter levels for the interaction term Day · Temp · O<sub>2</sub> in equation 9.7.

## 9.5 Modelling in practice: cured ham

When packaging cured meat products it is important to keep the O<sub>2</sub> and light exposure at a minimum. Møller *et al.* (2002) investigated the colour stability of cured ham under different packaging and storage conditions according to Table 9.3. Colour measurements were performed using a Minolta Colorimeter CR-300 (Minolta, Osaka, Japan). The effect of light was evaluated as the light intensity from a fluorescent tube measured on the package surface. The resulting regression model (after removal of insignificant effects) considering only two-factor interactions is shown in equation 9.8.

$$\begin{aligned}
 a^*\text{-value} = & \beta_0 + \beta_1 \cdot \text{ResO2} + \beta_2 \cdot \text{Vol} + \beta_3 \cdot \text{Light} + \beta_4 \cdot \text{Nit} + \\
 & \beta_5 \cdot \text{Time} + \beta_6 \cdot \text{MeasO2} + \beta_7 \cdot \text{ResO2} \cdot \text{Light} + \\
 & \beta_8 \cdot \text{ResO2} \cdot \text{Temp} + \beta_9 \cdot \text{ResO2} \cdot \text{MeasO2} + \\
 & \beta_{10} \cdot \text{Vol} \cdot \text{MeasO2} + \beta_{11} \cdot \text{Light} \cdot \text{MeasO2} + \\
 & \beta_{12} \cdot \text{Time} \cdot \text{MeasO2}
 \end{aligned} \tag{9.8}$$

where the  $\beta$ s are regression coefficients.

**Table 9.3** Packaging and storage conditions used in the models developed in Møller *et al.* (2002)

Modelling factor	Abbreviation	No. of levels	Setting of levels
Storage time (days)	Time	5	1, 3, 6, 9, 14
Residual O <sub>2</sub> level (%)	ResO2	3	0.1, 0.25, 0.5
Measured O <sub>2</sub> level (%)	MeasO2	–	Continuously
Oxygen transmission rate (ml/m <sup>2</sup> /24h/atm)	OTR	3	0.5, 10, 32
Volume ratio (product to headspace)	Vol	3	1:1, 1:3, 1:5
Light intensity (Lux)	Light	2	500, 1000
Nitrite content (ppm)	Nit	2	60, 150

As expected the  $a^*$ -value decreases with increased time, increased residual O<sub>2</sub> level, increased OTR, increased light intensity and decreased nitrite content. However, the study also shows the importance of interactions between factors. The interaction between O<sub>2</sub> level and product to headspace volume ratio is especially interesting. Normally, the focus is on the residual O<sub>2</sub> level (%) in the package and it is commonly overlooked that also the total amount of available oxygen molecules is important. The total amount of oxygen molecules available for colour deteriorative reactions is determined by the residual oxygen level after packaging, the meat to headspace volume ratio, and the amount of oxygen that permeates into the package headspace in combination. It is not sufficient to keep a low O<sub>2</sub> level in the package headspace. If the headspace volume is large there will still be plenty of oxygen molecules for colour deterioration.

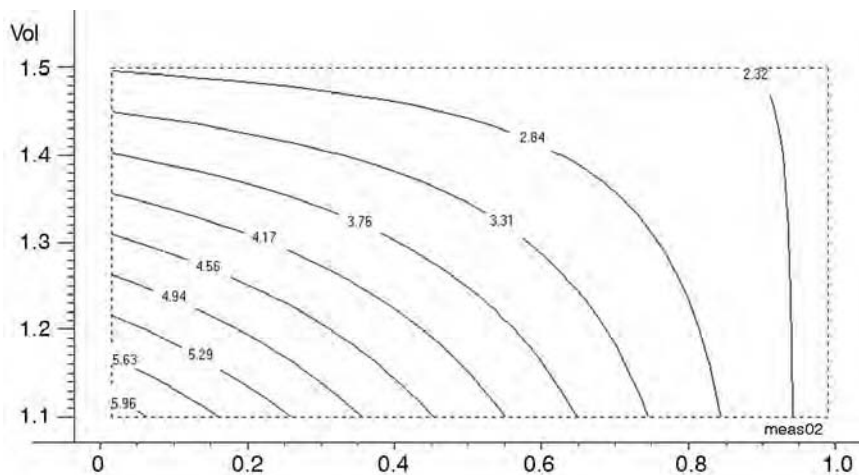
Figure 9.4 shows a contour plot of the interaction between ‘measured O<sub>2</sub> level’ and ‘volume ratio’ (the remaining factors are fixed to the following settings: residual O<sub>2</sub> level = 0.25%, illuminance = 1000 lux, nitrite = 60 ppm, storage time = 9 days). The  $a^*$ -value of the product for a given combination of ‘measured O<sub>2</sub> level’ and ‘volume ratio’ can be found from the plot by reading the  $a^*$ -value from the corresponding contour line, e.g., applying 0.10% ‘measured O<sub>2</sub> level’ and a ‘volume ratio’ of 1:1.3 results in an  $a^*$ -value of 5.6 after 9 days of storage. It appears that to maintain a high  $a^*$ -value it is necessary to keep both the oxygen level and the headspace volume low (lower left corner of the plot), solely keeping the O<sub>2</sub> level low is not sufficient. The interaction between O<sub>2</sub> level and light intensity is also important. In order to maintain a good product colour it is necessary simultaneously to keep both the O<sub>2</sub> level and the light intensity low (Møller *et al.*, 2002).

## 9.6 Internal factors affecting colour stability

### 9.6.1 Fresh meat

Large variations in colour stability between meat of different origins can strongly influence the developed models. Different meat types show large





**Fig. 9.4** Contour plot of the interaction effect between volume ratio (product:headspace) and measured  $O_2$  level (%) after nine days storage (adapted from Møller *et al.*, 2002).

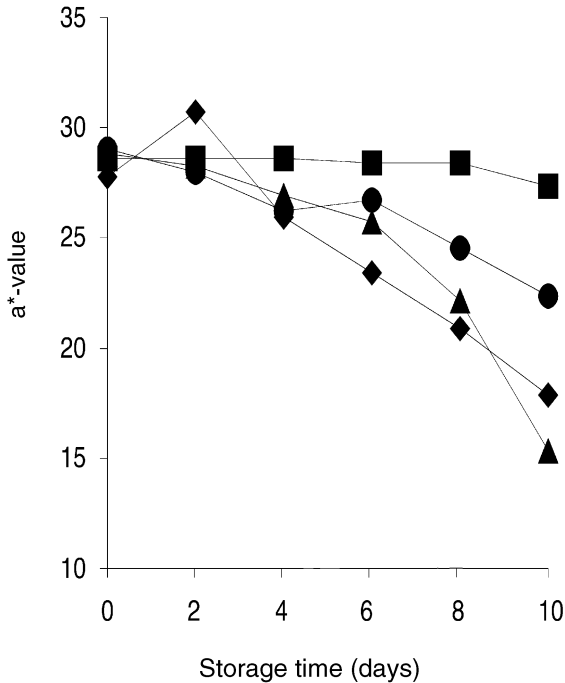
variability due to different myoglobin content and different metabolic type (Renner, 1990). The content of myoglobin is, e.g., largest in beef followed by lamb and pork, and the colour of pork is more stable than the two other species. Steaks of *Longissimus dorsi* muscles have high colour stability and steaks of *Semi-membranosus* muscles have medium colour stability. Animals of different age, breed, feeding, etc., will also show differences in colour stability (Renner, 1990; Jensen *et al.*, 1998)

It appears from Figs. 9.5 and 9.6 that there is a huge variation in colour stability between meat from different sources. A range of intrinsic factors influence the oxidative balance in raw meat and thereby the colour stability of the meat (Bertelsen *et al.*, 2000). Thus the oxidative stability of muscles is dependent on the composition, concentrations, and reactivity of (i) oxidation substrates (lipids, protein and pigments), (ii) oxidation catalysts (prooxidants such as transition metals and various enzymes) and (iii) antioxidants, e.g., vitamin E and various enzymes. For review see Bertelsen *et al.* (2000).

Meat from different sources shows different tendencies to undergo colour deterioration. It is therefore necessary to investigate meat from a large number of sources to be able to make general conclusions. Despite the large variations in the colour stability of meat from the different animals and muscle types investigated in Section 9.4, the pronounced effect of temperature and the constant interval of  $O_2$  are common. Only the rate of colour deterioration differs.

### 9.6.2 Cured meat

A range of intrinsic factors affect the colour stability of nitrite-cured meat products. The most important are the level of nitrite and the content of vitamin E

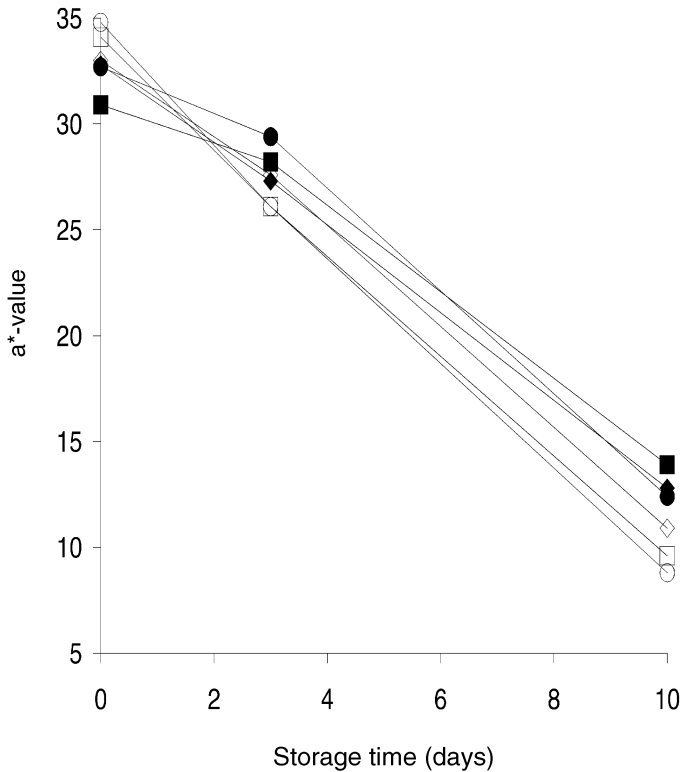


**Fig. 9.5** Measured  $a^*$ -values for four different animals stored in 80%  $O_2$  at 8°C (adapted from Jakobsen and Bertelsen, 2000).

(Weber *et al.*, 1999). Thus, optimum colour stability can be achieved only by using a multifactorial approach, where both intrinsic and extrinsic factors are considered (Bertelsen *et al.*, 2000). From Fig. 9.7 the effect of the nitrite content on the rate of colour deterioration is evident. Increasing the nitrite content stabilises the colour. This result emphasises the necessity of investigating the specific product of interest in order to define critical levels of packaging and storage factors.

## 9.7 Validation of models

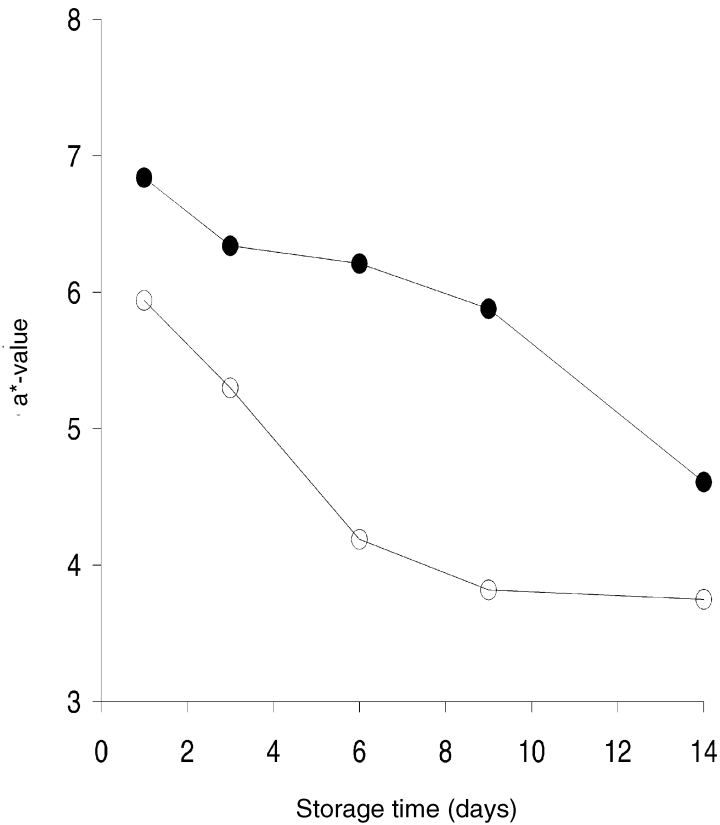
The examples in Sections 9.4 and 9.5 clearly demonstrate the usefulness of modelling for identification of the most important factors/interaction of factors affecting colour deterioration. They also demonstrate how critical limits/intervals of these factors can be identified. For fresh beef it is recognised that keeping a low storage temperature is the key parameter to obtain a long colour shelf life. In addition a wide interval of oxygen partial pressure exists that result in optimal colour stability, leaving the possibility of optimising the gas composition with respect to other quality deteriorating reactions, e.g., lipid oxidation without compromising the colour stability. With respect to cured meat



**Fig. 9.6** Measured  $a^*$ -values for two muscle types from three different animals, stored in 80%  $O_2$  at 8°C. *Longissimus dorsi* muscles (closed symbols) and *Semi-membranosus* muscles (open symbols).

products it is important to realise that several factors influence the total amount of  $O_2$  molecules available for oxidation.

Modelling of MAP systems shows great potential for optimising/tailoring storage and packaging parameters to maintain product quality, in this case a good meat colour stability (Jakobsen and Bertelsen, 2000; Lyijynen *et al.*, 1998; Pfeiffer *et al.*, 1999). As shown, modelling can be used to identify the more important factors affecting quality loss and to define critical levels of these factors. Multivariable experimental design is necessary to be able to investigate the large number of influencing factors on several levels as well as the interactions between factors. However, due to large biological differences between meat from different sources and to differences in handling and processing of the meat it is also important to recognise that internal factors have an effect on the developed models. The described models can be used to predict the general response of a meat product to changes in external factors, but not to predict the exact  $a^*$ -value for a certain piece of meat. That would require much more specific models (for each product type) and incorporation



**Fig. 9.7** Measured  $a^*$ -values for cured ham containing 150 ppm (filled circles) and 60 ppm (open circles) nitrite (each point is an average of 42 samples) (data from Møller *et al.*, 2002).

of knowledge on the internal factors into the models (Jakobsen and Bertelsen, 2000).

## 9.8 Future trends

The obvious tools for optimisation of product shelf life through controlling the packaging and storage conditions are computer simulations. Models of changes in headspace gas composition should be combined with models describing changes in the most important quality parameters. A computer program should be given inputs on:

- permeability of the different packaging films to be compared
- storage temperature
- relative humidity during storage

- gas composition measured after packaging
- the headspace and product volumes
- light conditions during storage.

By using computer simulations the time for reaching, e.g., an oxygen content critical for the colour stability of a given product can be predicted. Furthermore, the permeability characteristics of the packaging material can be set, or the shelf life using a specific packaging film can be predicted. Such computer simulations were developed by Pfeiffer *et al.* (1999) primarily for predicting quality changes, moisture gain and lipid oxidation in several dry products. The models described in the earlier sections are well suited for defining critical factors and levels for maintaining good colour stability of fresh and cured meat products.

Computer simulations are an attractive supplement to storage experiments since it will not be necessary to test all combinations of the factors before the optimal packaging and storage conditions can be chosen considering both the product shelf life and minimisation of the packaging material.

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# 10

## Analysing changes in fruit pigments

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### 10.1 Introduction

Colours in fruits and vegetables are mainly due to three families of pigments, chlorophylls, carotenoids and anthocyanins, responsible for green, red-yellow and red to blue-purple colours, respectively. The main role of these pigments in fruit, through the external colour of products, is the attraction of insects and animals in order to ensure the dissemination of seeds. In the same way humans are attracted to, and frequently fascinated by, fruit colour. In fact fruit colour perception is the first criterion in determining the acceptability of any kind of fruit and in general of any food. For this reason, knowledge of the biochemical and physiological processes of biosynthesis and catabolism of plant pigments is fundamental to understanding the mechanisms responsible for the different pigments in fruits. It would be useful to know if this composition could change depending on the physiological stage of fruit. In addition, detailed knowledge of fruit pigment composition will allow evaluation of the effects of postharvest treatments in retaining colour and quality and extending the shelf life of fruits and vegetables and derived products. In canned, frozen or minimally processed juice or marmalades it will be crucial to know the main factors affecting pigment stability as well as the main changes associated with the type of processing. For example, in the case of ripening mature olives the external colour is basically due to anthocyanins but the masked chlorophylls and carotenoids are responsible for the olive oil colour and in a great measure its quality (Mínguez-Mosquera *et al.* 1991a). Analysing pigment composition of fruit and vegetables and their derivatives, not only is it interesting to determine their genuineness (e.g., flavonoids and carotenoids), but also for optimising postharvest treatments during harvesting, storage and transport as well as during industrial

transformation (Artés *et al.* 1998a, 2000a,b; Gandul-Rojas and Mínguez-Mosquera 1996; Gandul-Rojas *et al.* 2000; García-Viguera *et al.* 1993, 1998; Gil *et al.* 1995c, 1996a,b, 1998; Mínguez-Mosquera and Hornero-Méndez, 1994a; Mouly *et al.* 1999).

## 10.2 Pigments in fruits: chlorophylls, carotenoids and anthocyanins

### 10.2.1 Chlorophylls

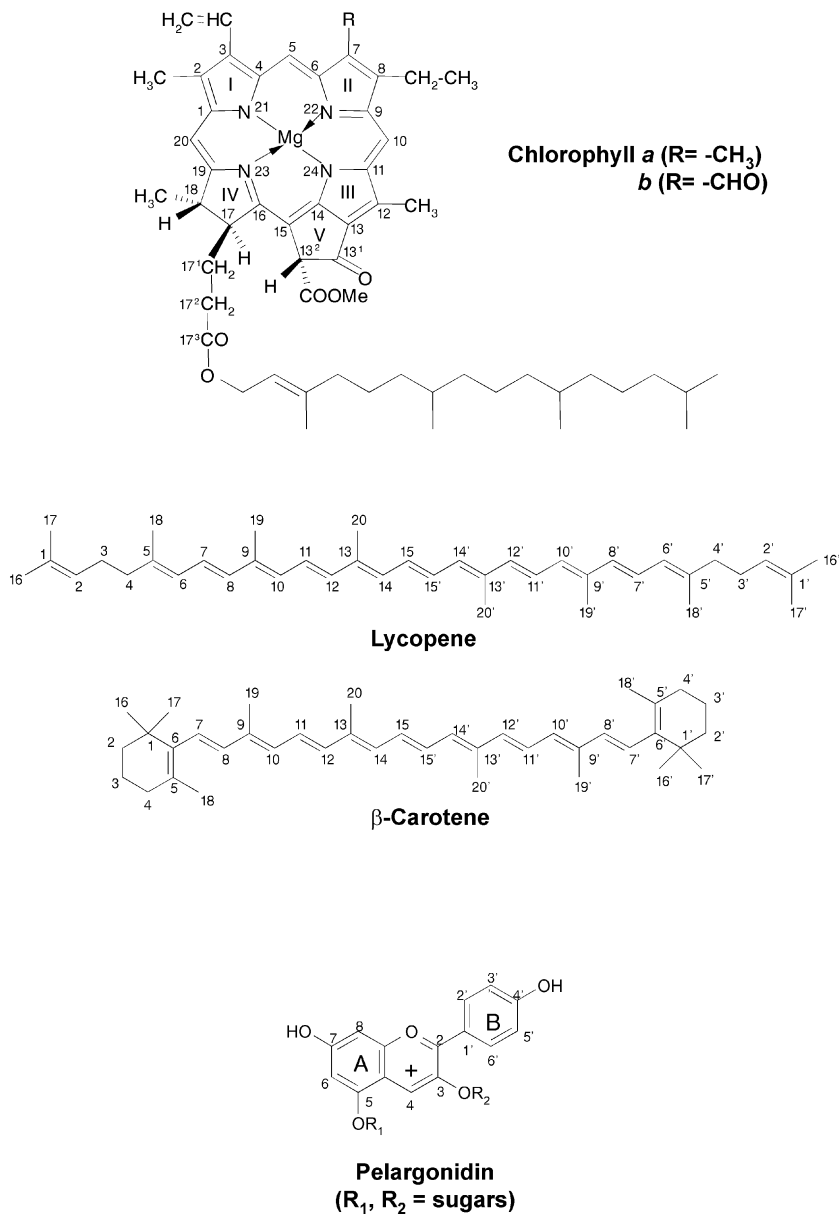
The green colour in all higher plants, including fruits, is due to chlorophylls, which participate in photosynthesis, one of the most important life processes, converting energy from light into chemical energy. Chlorophylls are chemically classified within the porphyrin group with a common structure comprising four units of pyrrole, a tetrapyrrole macrocycle, linked by methine bridges and coordinated with a  $Mg^{2+}$  ion. Chlorophyll also contains a modified propionic acid chain in the form of cyclic  $\beta$ -ketoester (isocyclic ring) and, on C-17, a chain of propionic acid esterified with the diterpene alcohol phytol, making the molecule lipophilic. In higher plants there are two types of chlorophyll, *a* and *b*. The difference between them is that chlorophyll *a* has a methyl group on carbon C-7, whereas chlorophyll *b* has an aldehyde group (Fig. 10.1). In higher plants the ratio of chlorophyll *a* to *b* (Chl *a/b*) commonly varies between 3 and 1, depending on a multitude of factors – both genetic (species, variety, etc.) and environmental (luminosity, water stress, mineral nutrition, etc.) (Lichtenthaler 1968). Thus, for instance, plants exposed to the sun tend to have a higher Chl *a/b* ratio than plants in the shade.

The chlorophyll pigments of higher plants are located in specialised plastids named chloroplasts. Chloroplasts have a system of double membranes in the form of sacs – the thylakoids – which contain all the chlorophyll in association with proteins. The amphoteric nature of the chlorophylls allows the phytol chain to be embedded in the thylakoid membrane, with the porphyrin ring linked by non-covalent bonds to proteins (Heaton and Marangoni 1996, Salisbury and Ross 1985), constituting supramolecular structures known as *photosystems*. In higher plants, there are always two photosystems, PSI and PSII, with PSII being responsible for photolysis of water, and PSI for the reduction of  $NADP^+$ . It is noteworthy that chlorophyll *b* is found mainly in PSII, exclusive to organisms with oxygenic photosynthesis, and evolutionally more developed (Gross 1987).

### 10.2.2 Carotenoids

Among the pigments present in living organisms, there is no doubt that carotenoids are, after chlorophylls, the most widely distributed in nature. They are found throughout the plant kingdom – in both photosynthetic and non-photosynthetic tissues in bacteria, in fungi, and in animals, although the latter





**Fig. 10.1** Structures for chlorophyll *a*, chlorophyll *b*, lycopene,  $\beta$ -carotene and pelargonidin.

are unable to synthesise them and so incorporate them from dietary plants. It is estimated that the annual production of carotenoids in nature is around  $10^8$  tons (Weedon 1971). In 1831, Wackenroder isolated an orange pigment from the carrot (*Daucus carota*), and coined the term *carotene* from the Latin word

*carota*. Later, in 1837, Berzelius assigned the name *xanthophylls* to the yellow pigment of autumn leaves. Today more than 650 different carotenoids have been isolated from natural sources and identified, and more than 100 have been found in fruit and vegetables (Britton and Hornero-Méndez 1997).

Carotenoids are tetraterpenes derived from a symmetrical C40 skeleton. Carotenoids can be classified into two great groups: *carotenes*, which are strictly hydrocarbons, and *xanthophylls*, derived from the former that contain oxygenated functions. Structurally, the carotenoids may be acyclic (e.g., lycopene) or contain a ring of five or six carbons at one or both ends of the molecule (e.g.,  $\beta$ -carotene). Figure 10.1 shows the structure and the system of numbering using lycopene and  $\beta$ -carotene as models of acyclic and bicyclic carotenoids respectively. Structures of some representative carotenes and xanthophylls, commonly found in fruits are also illustrated.

In plants, the carotenoids are located and accumulated in specialised subcellular organelles called plastids, concretely in the chloroplasts – accompanying chlorophylls – and chromoplasts (Goodwin and Britton 1988). The chloroplasts are present in all photosynthetic tissues (mainly leaves but also in immature fruits), where practically all the carotenoids are present in the form of chlorophyll-carotenoid-protein complexes (photosystems) at the level of the thylakoid membranes. In this environment, the carotenoids have their prime natural function as assistant collectors of light energy (antenna pigments) in the photosynthetic process, since, due to their absorption spectrum they are able to capture photons that escape the reach of the chlorophylls. Nevertheless, the chromoplasts present in ripe fruits, flowers, and certain roots and tubercles are the organelles specialising in the massive accumulation of carotenoids, and have the greatest variety of structural forms. In the case of chromoplasts, the carotenoids are usually accumulated in lipid-rich structures, the plastoglobules, as for example in the fruits of the genus *Capsicum* and many flowers. In certain cases, such as tomato (*Lycopersicon esculentum*), carrot, and pumpkin (*Cucurbita maxima*), the presence has also been reported of carotenoid crystals, mainly carotenes, immersed in the stromatic space (Sitte *et al.* 1980).

Details of chromoplast ultrastructure and the location of the carotenoids have been extensively reported (e.g., Gross 1987; Kirk and Tilney-Bassett 1978; Sitte *et al.* 1980). Four major types of structure have been identified by electron microscopy, namely globulous, tubulous, membranous and crystallous (Sitte *et al.* 1980). The change from chloroplast to chromoplast, which is associated with fruit ripening, is especially important in the case of fruits denominated *carotenogenic* (e.g., pepper and tomato), characterised by a massive synthesis of carotenoids during ripening, usually accompanied by a change in the carotenoid profile of the fruit. It is noteworthy that the chromoplast xanthophylls are usually esterified with different fatty acids, increasing their lipophilic character and facilitating their accumulation in the plastoglobules. In fruits and flowers, the main function of carotenoids is undoubtedly to attract animals (insects, birds, and mammals) so that they assist in seed dispersion and pollen transport (Bartley and Scolnik 1995). Carotenoids also play a very important role as protectors of the chlorophylls and

the photosynthetic apparatus in general (the quenching effect) by blocking very reactive forms of triplet chlorophylls ( $^3\text{Chl}$ ) and singlet oxygen ( $^1\text{O}_2$ ) formed during the capture of light energy. They also take an active part in the plant's photoprotective and antioxidant action (Frank and Cogdell 1993).

### 10.2.3 Anthocyanins

In plant tissues a series of secondary metabolites including phenolic compounds are commonly present. These phenolic metabolites are very variable in chemical nature and biological properties and play a significant role in fruit and vegetables due to their participation in several quality attributes such as appearance (mainly colour), taste and flavour, as well as in their health-promoting properties (Tomás-B. and Espín 2001). Among the different phenolic compound metabolites that are relevant in plant foods, flavonoids represent the largest class. Coloured flavonoids are synthesised by almost all flowering vegetables and can be found in all plant organs. They are also found either unaltered or modified in plant food products and they constitute a regular component in animal and human diet (about 10 g/day by an adult) (Brouillard *et al.* 1997). Anthocyanins are the most noticeable group among flavonoids and are relevant to different functions within plants. In fact the presence of anthocyanins in petals is intended to attract pollinators and to aid in seed dispersal. On the other hand, anthocyanins and other flavonoids can also be important as antioxidants, feeding deterrents and as protection against damage from UV irradiation (Holton and Cornish 1995).

### 10.2.4 Nature and occurrence of anthocyanins

Some of these phenolic compounds are directly related to colours in plants, and in particular anthocyanidins are natural pigments present as anthocyanins in plant tissues in glucoside forms with a C6-C3-C6 skeleton. Only a few phenolic compounds absorb light in the visible range and anthocyanins are the only significant subgroup of polyphenols visible to the human eye (Brouillard *et al.* 1997).

Anthocyanins are the most important group of water-soluble pigments in plant tissues, and produce red, blue, purple and black, and intermediate colours of many fruits and vegetables and their derived foods. Anthocyanins are dissolute in fruit and vegetable vacuolar juice, mainly in mature epidermal cells (apple, apricot, artichoke, asparagus, eggplant, fig, red-lettuce, nectarine, red-onion, peach, pear, plum, pomegranate, red-skinned potato, radish, etc.), although they can also accumulate in flesh tissues (apple, blackberry, blueberry, red-carrot, cherry, cranberry, red- and black-currant, fig, grape, peach, plum, pomegranate, olive, 'Sanguine' orange, purple sweet potato, raspberry, strawberry, etc.) (Clifford 2000; Tomás-B. and Espín 2001).

There are six common variations in the number and position of the hydroxyl and methoxyl substituents on the basic anthocyanidin skeleton (Fig. 10.1 shows

an example of the pelargonidin aglycone with two different sugar residues), although several hundred anthocyanins are presently known to vary also on the identity, number and position at which sugars (mainly glucose, galactose, rhamnose and arabinose) are attached to the skeleton, and the extent of sugars acylation and the identity of the acylating agents (Clifford 2000).

Colour due to anthocyanins is notably unstable, especially due to enzymic hydrolysis in harvested products. On the other hand, anthocyanins are rapidly oxidised in the vacuole of plant cells in the presence of molecular O<sub>2</sub> by the action of tyrosinase or polyphenol oxidase (PPO; EC 1.14.18.1), a copper-containing enzyme located in the cell cytoplasm and mainly associated to the plastids membrane. But anthocyanins are not direct substrates for PPO, although when possessing a dihydroxy B-ring they can suffer coupled oxidations with quinones formed by the action of PPO on other phenols. PPO catalyses the hydroxylation of monophenols to o-diphenols (cresolase activity) and the oxidation of o-diphenols to o-quinones (catecholase activity). These o-quinones are very reactive molecules that rapidly condense by combining with amino or sulfhydryl groups of proteins and with reducing sugars, producing different brown, black or red polymers of high molecular weight and unknown structure. These are usually referred to as melanins. It has been reported that peroxidases (POD; EC 1.11.1.7.) could also be involved in enzymatic browning of plant foods. These oxidations could be prevented by the ascorbic acid (vit. C), and development of browning is a symptom of vit. C disappearance, and loss of nutritional value that could be important after processing. As it is generally considered that an increase in anthocyanin content is a favourable quality attribute, this deterioration of original substrates has a great visual impact commonly reducing the commercial quality, the organoleptic acceptance and the nutritional value of the products. There are some exceptions, for example, dehydrated plums and grapes, date, fig or black tea (Artés *et al.* 1998a; Clifford 2000; Sánchez-Ferrer *et al.* 1995).

Processing for industrial and home-made preparation of plant food containing anthocyanins led to an undesirable yellowish and brownish pigment due to instability of anthocyanins once removed from their natural environment and the protection provided by copigmentation. Peeling, cutting, slicing, etc., disrupts cell membranes and allows the mixing of enzymes and substrates previously in separated cellular compartments (Clifford 2000). On the other hand the degradative effect of vit. C on anthocyanin stability leading to colour alteration has been demonstrated in model solutions and in natural pomegranate juice systems which show a high antioxidant activity. Consequently, the addition of vit. C to pomegranate juice to prevent browning and as an extra source of vit. C, reduces the concentration of anthocyanins without any particular benefit in colour retention (Martí *et al.* 2001). The degradation of anthocyanins is also affected by their exposure to light and heat although they are much more stable than anthocyanidins with respect to light and temperature. Glucosylation provides protection against photodegradation and the formation of intermolecular copigmentation complexes and ion-pairs contributes to reduce these degradative reactions (Brouillard *et al.* 1997).

### 10.3 Categorising fruits by pigment composition

Although chlorophylls, carotenoids and anthocyanins are responsible for fruit colour, there is not a single pattern for pigment composition, which will be determined by the biosynthetic and degradative pathways that predominate during the pre- and postharvest ripening of fruits. The study and analysis of pigment composition in fruit makes it possible to distinguish between different kind of fruits. In some cases it is possible to find members of the three pigment families in a ripe fruit, but in most cases only one or two kinds of pigments are found. Apart from unripe fruits, which owe their green colour to the existence of the chloroplastic pigments (chlorophylls and carotenoids), the ripe fruits may be classified into four groups according to their pigment composition:

1. Fruits with a total disappearance of chlorophylls, usually having a characteristic yellow colour due to unmasked carotenes and xanthophylls following the degreening process (i.e., banana, plantain).
2. Fruits with a marked *de novo* biosynthesis of carotenoids, referred to as *carotenogenic* fruits (i.e., tomato, red pepper, orange, persimmon, etc.).
3. Fruits with a marked *de novo* biosynthesis of anthocyanins (i.e., grape, apple, olive, pomegranate, red cherry, raspberry, cranberry, etc.).
4. Fruits with retention of chlorophylls after ripening (i.e., kiwifruit, avocado, chlorophyll retaining cultivars of red pepper, green fleshed tomato, etc.).

The distribution of carotenoids among the different groups of higher plants does not follow a single pattern. In green plant tissues, the class and content of carotenoid pigments follows the general model associated with the presence of chloroplasts, with  $\beta$ -carotene being the predominant carotene (25–30% of the total carotenoids), followed by a set of xanthophylls, lutein (40–45%), violaxanthin (10–15%), and neoxanthin (10–15%) contained in the light-harvesting antenna complexes. Zeaxanthin,  $\beta$ -cryptoxanthin, and antheraxanthin are also present in significant amounts under appropriate conditions as components of the photoprotective xanthophyll cycle (Demmig-Adams and Adams 1993).

In fruits and other non-green tissues such as flowers, the xanthophylls are normally found in greater amounts. The carotenoids of fruits, however, show much greater diversity, and exotic structures may be found. Apocarotenoids, in which one end group has been removed, are common in fruit (e.g.,  $\beta$ -citraurin in *citrus*). The carotenoids in ripe fruit are usually different from those that are present in the unripe, green fruit. The carotenoid compositions can be very complex; in *Capsicum annuum* more than 25 carotenoids have been detected by HPLC and many of these identified (Matus *et al.* 1991; Mínguez-Mosquera and Hornero-Méndez 1993). When acyl esters and geometrical isomers are included, the complexity can be daunting (Mínguez-Mosquera and Hornero Méndez 1994b). Exceptionally, in mango (*Mangifera indica*) and persimmon (*Diospyros kaki*),  $\beta$ -cryptoxanthin and zeaxanthin are the major pigments. In contrast, in tomato (*Lycopersicon esculentum*) the major carotenoid is lycopene, a carotene.

In certain fruits, a carotenoid, besides being the major one, is limited totally or almost totally to a single plant species. Capsanthin and capsorubin are found almost exclusively in ripe fruits of *Capsicum*, and are responsible for their attractive red colour (Davies *et al.* 1970; Goodwin 1976; Goodwin and Goad 1970; Mínguez-Mosquera and Hornero-Méndez 1994c).

The orange fruit (*Citrus sinensis*) contains varying amounts of  $\beta$ -citraurin and  $\beta$ -citraxanthin (both apocarotenoids), together with  $\beta$ -cryptoxanthin, lutein, antheraxanthin, violaxanthin, and traces of their carotene precursors (Farin *et al.* 1983). The presence and distribution of the most common carotenoid pigments found in fruit and vegetables, and in general in foods, are shown in Table 10.1. As a simplification, the carotenoid compositions of fruit have been classified into several groups (Table 10.2) (Goodwin and Britton 1988). Although the distinctions are not always clear, this broad classification remains useful and can now be rationalised on the basis of the presence or absence of particular genes for carotenoid biosynthesis (Cunningham and Gantt 1998). Goodwin (1980) and Gross (1987, 1991) extensively reviewed detailed accounts of the occurrence of carotenoids in fruit and vegetables presenting tabulated data on carotenoid compositions.

**Table 10.1** Fruit and vegetable occurrence of some common carotenes and xanthophylls

Carotenoid	Occurrence
<b>Carotenes</b>	
$\alpha$ -Carotene, $\beta$ -carotene, $\delta$ -carotene, $\gamma$ -carotene, $\epsilon$ -carotene, $\zeta$ -carotene	Most fruits and vegetables, especially in carrots, sweet potato, palm tree fruit. Delta tomato mutant has $\delta$ -carotene as its major carotene. Rose hips are a good source of $\gamma$ -carotene
Lycopene, neurosporene	Tomato ( <i>Lycopersicon esculentum</i> ), water melon and rose hips ( <i>Rosa</i> spp.)
Phytofluene, phytoene	Carotenoid-rich fruits, flowers and roots (carrot)
<b>Xanthophylls</b>	
Antheraxanthin	Anthers and petals of many yellow flowers, also in fruits and vegetables
Bixin, norbixin	Annatto ( <i>Bixa orellana</i> ) seeds
Capsanthin, capsanthin-5,6-epoxide, capsorubin	<i>Capsicum annuum</i> ripe fruits
Crocetin	Saffron ( <i>Crocus sativus</i> ) flowers
Cucurbitaxanthin A	Pumpkin ( <i>Cucurbita maxima</i> ) flesh
Lactucaxanthin	Lettuce ( <i>Lactuca sativa</i> ) leaves
Lutein, violaxanthin, neoxanthin	Green fruits, vegetables, and flowers
Luteoxanthin, neochrome, auroxanthin	Vegetables and fruits processed under acid conditions and fermentation
Rubixanthin	Rose hips ( <i>Rosa</i> spp.)
Zeaxanthin, $\beta$ -cryptoxanthin, $\alpha$ -cryptoxanthin, cryptoxanthin-5,6-epoxide	Seeds (corn), flowers and fruits: mango, papaya, persimmon.

**Table 10.2** General groups into which fruit can be classified on the basis of their carotenoid compositions (adapted from Goodwin and Britton 1988)

Group	Pigment pattern
I	Insignificant amounts
II	Small amounts generally of chloroplast carotenoids
III	Relatively large amounts of lycopene and its hydroxy derivatives
IV	Relatively large amounts of $\beta$ , $\beta$ -carotene and its hydroxy derivatives
V	Large amounts of epoxides, particularly furanoid epoxides
VI	Unusual carotenoids, e.g., capsanthin
VII	Poly-Z carotenoids, e.g., prolycopene
VIII	Apocarotenoids, e.g., $\beta$ -citraurin

In green plant tissues, the xanthophylls are of free form. However, as a consequence of leaf senescence and ripening of many fruits, coinciding with the transformation of the chloroplasts into chromoplasts, the carotenoid pigments undergo esterification with different fatty acids. The esterification is related to the ability of the plant (in particular fruits and flowers) to overproduce and accumulate carotenoid pigments. Changes in the esterification profile of the xanthophylls in red pepper fruit has been proposed as a maturity index (Hornero-Méndez and Mínguez-Mosquera 2000). The process of esterification, which does not affect the chromophore properties of the pigment, seems to be related to a higher capacity for overaccumulation of carotenoids in the case of fruits, and therefore a stronger attraction for seed-dissemination vectors to increase successful reproduction (Bartley and Scolnick 1995). Xanthophyll esterification is always related with *de novo* carotenogenesis in ripening fruits. This fact has been used to prove an unusual carotenogenesis in olive fruit of the 'Arbequina' cultivar which, as in the other olive cultivars shows a marked anthocyanic biosynthesis during ripening (Roca and Mínguez-Mosquera 2001a). This finding is important since esterified xanthophyll will pass lipid matrix of the extracted virgin olive oil more efficiently.

The occurrence of anthocyanins in fruits depends on the kind of fruit, cultivar, structure of tissues, geographical location, position of the fruit on the tree, and cultivation conditions. As an example, pomegranate (*Punica granatum*, *Punicaceae*) at commercial maturity stage shows a high total anthocyanins content ranging from 50 to 267 mg/kg fresh weight of arils for the sweet 'Mollar' and 'Valenciana', sour-sweet 'Pinón Tierno de Ojós' and sour 'Blanca de Albaterra' Spanish cultivars. The lowest amount of total anthocyanins was found in the sour cultivar, that showed the more stable anthocyanins, since these pigments are stabilised at pH values below 3 (Artés *et al.* 1998b; Gil *et al.* 1995a; Hernández *et al.* 1999). Exceptionally, in a particularly very severe drought season, 'Mollar' cultivar showed a total amount of anthocyanins of 389 mg/l juice (Gil *et al.* 1996c). In Tunisian pomegranate juices the total anthocyanins range was 6 to 120 mg/l (Gil *et al.* 1995c). Total anthocyanins in minimally freshly processed pomegranate seeds was around 185 mg/l in

‘Mollar’ cultivar (Gil *et al.* 1996a) and 200 mg/l in ‘Wonderful’ cultivar grown in California (Holcroft *et al.* 1998). The profile of anthocyanins in the juice of pomegranate shows six components: delphinidin 3-glucoside and 3,5-diglucoside, cyanidin 3-glucoside and 3,5-diglucoside, and pelargonidin 3-glucoside and 3,5-diglucoside. However, in the fruit husk only the cyanidin and pelargonidin derivatives are present (Du *et al.* 1975; Gil *et al.* 1995b).

The total amount of anthocyanins in the juices obtained from seeds of ‘Mollar’ cultivar fruits picked on the external part of the tree, which show a reddish husk, are usually 60% lower than in those from fruits of the internal part, which show a yellowish husk (Gil *et al.* 1995b). It has been also found that pomegranates growing in soils with poor nutrient composition and with high salt concentrations produced a lesser anthocyanins content than those cultivated in more fertile soils, although the same profile and relative amounts of anthocyanins were found (Gil *et al.* 1995a).

Pelargonidin 3-glucoside, pelargonidin 3-rutinoside and cyanidin 3-glucoside are the anthocyanins responsible for strawberry (*Fragaria x ananassa*) colour. Intercultivar differences in total anthocyanins as well as in pigment degradation have been found throughout storage at 20, 30 and 37 °C of strawberry jams made with ‘Oso Grande’, ‘Tudla’ and ‘Chandler’ cultivars. ‘Oso Grande’ jam had the lowest anthocyanins concentration (110 mg/g fresh weight), higher monomeric pigment degradation during processing and storage and shortest shelf life. The highest initial anthocyanins concentration was found in ‘Chandler’ jam (195 mg/g fresh weight) but similar reactions during processing and storage occurred between ‘Chandler’ and ‘Tudla’ jams (García-Viguera *et al.* 1999).

## 10.4 The formation and transformation of pigments during fruit development and ripening

The characteristic green colour of unripe fruits, apart from leaves, is due to the presence of chlorophylls and carotenoids. However when ripening starts, many complex biochemical changes take place, including pigment composition and colour changes that involve both biosynthetic and catabolic processes. During ripening the thylakoids are reorganised and chloroplasts are gradually replaced by a chromoplast containing only carotenoids. In some fruits such as avocado (*Persea americana*) and kiwifruit (*Actinidia chinensis*) chlorophylls are retained in the pulp of the ripe fruit. However this is unusual and in most fruits carotenoids become unmasked when chlorophylls disappear upon ripening and usually this is accompanied by a marked *de novo* biosynthesis of carotenoids. In many fruits ripening is also associated with an intense biosynthesis of the water-soluble anthocyanins which are accumulated in the central vacuoles of the mesophyll cells.

Although all these colour and pigment composition variations occur at the same time, very different biochemical pathways are involved for each class of pigment, chlorophylls, carotenoids and anthocyanins. Usually chloroplastic



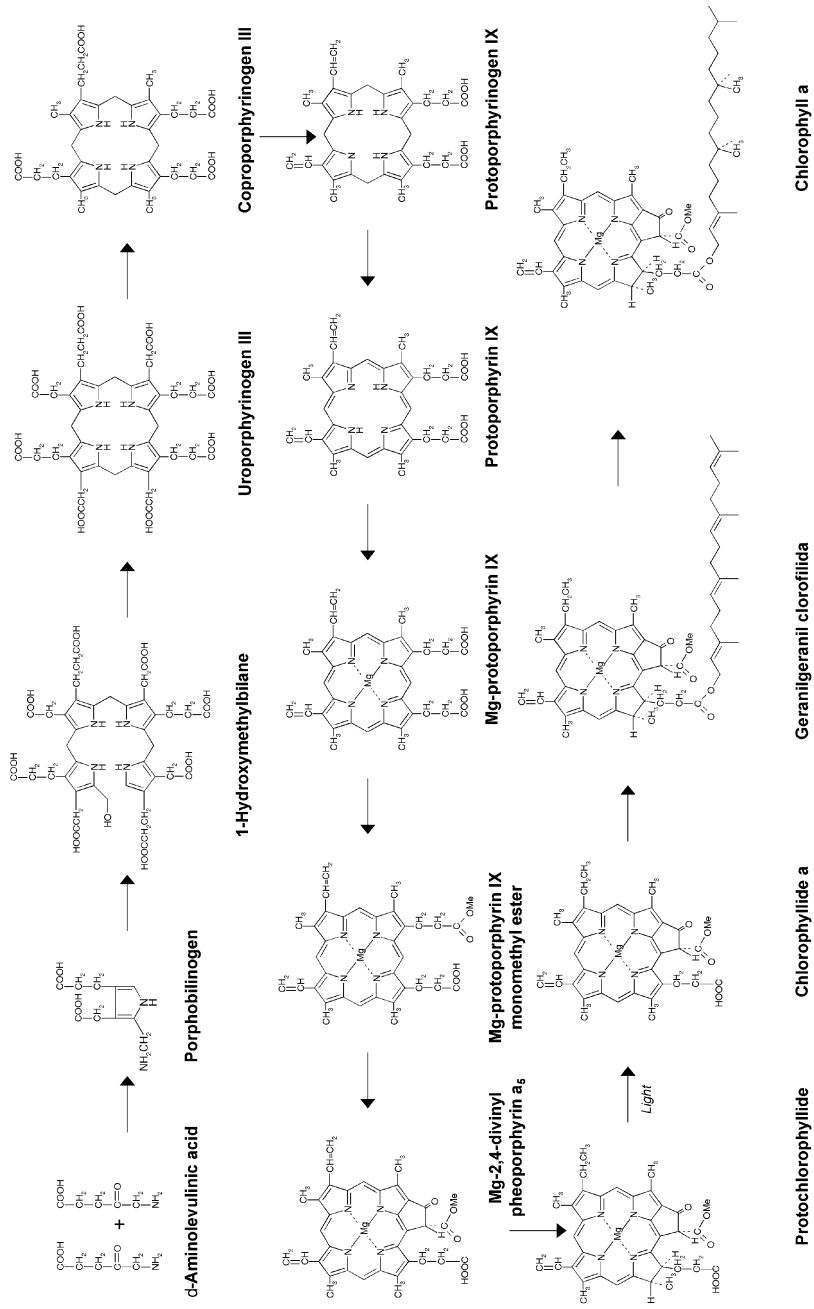
pigment biosynthesis is observed during fruit development, which stops with the onset of ripening and from this point chlorophyll catabolism and *de novo* carotenoid and/or anthocyanins biosynthesis are implicated.

## 10.5 Chlorophylls

### 10.5.1 Chlorophyll biosynthesis

Chlorophyll is formed following the same basic pathway used for all living organisms to produce porphyrins. The chlorophyll biosynthesis pathway has been well investigated but still some details remain unknown (Von Wettstein *et al.* 1995), although a general scheme proposed by Granick in the 1950s is accepted (Fig. 10.2). For a better understanding of the biochemical changes involved in chlorophyll formation it is convenient to consider different stages: (i) formation of the  $\delta$ -aminolaevulinic acid (ALA); (ii) formation of the monopyrrole (porphobilinogen); (iii) formation of uroporphyrinogen (the first tetrapyrrole macrocycle); (iv) formation of protoporphyrinogen by sidechain reaction; (v) formation of protoporphyrin IX by dehydrogenation of the macrocycle; (vi) chelation with  $Mg^{2+}$  to give magnesium protoporphyrin IX; (vii) formation of the isocyclic ring and protochlorophyllide a; (viii) reduction of protochlorophyllide to chlorophyllide and its esterification; (ix) formation of chlorophyll a; (x) biosynthesis of chlorophyll b.

The synthesis of ALA may be produced by at least two alternative routes, although in higher plants and algae, ALA is mainly formed by transamination of an amino acid as L-alanine to  $\gamma,\delta$ -dioxovaleric acid by the ALA transaminase. Next, two molecules of ALA are asymmetrically condensed to give the porphobilinogen reaction that is carried out by the porphobilinogen synthetase. The formation of the first tetrapyrrole (uroporphyrinogen III) is done by head-to-tail condensation of four porphobilinogen molecules. This reaction is complex and involves the formation of a linear bilane by assembling the four monopyrrole units, which are rapidly cyclised to uroporphyrinogen III. The next step is the formation of protoporphyrinogen IX by the sequential decarboxylation of the four acetic acid sidechains of uroporphyrinogen III to methyl groups, and the decarboxylation of two propionic acid sidechains of the coproporphyrinogen III intermediate. Protoporphyrinogen IX is now converted to protoporphyrin IX by dehydrogenation, this being the last intermediate process common to both chlorophyll and haem biosynthesis. The following step is the first specific stage in the chlorophyll biosynthesis and involves the chelation of the macrocycle with the magnesium ion to form Mg protoporphyrin IX that is catalysed by the Mg protoporphyrin IX chelatase. Following chelation, methylation of the propionic acid sidechain at C-13 takes place giving the Mg protoporphyrin IX monomethyl ester. This methylated propionic acid residue is used to form the isocyclic ring (ring V) by cyclisation and  $\beta$ -oxidation reactions to give the Mg-2,4-divinyl-pheoporphyrin a5, which is later reduced at the vinyl group in C-8 to form protochlorophyllide a. The conversion of proto-



**Fig. 10.2** Chlorophyll biosynthetic pathway.

chlorophyllide a into chlorophyll a is a photoreduction of the double bond at C-17 of the ring IV. This step is also known as the 'greening stage' since the chlorophyllide contains the chromophore responsible for the green colour of chlorophyll a. The next and final step in the biosynthesis of chlorophyll a includes the esterification of the C-17 propionic acid residue at the ring IV of chlorophyllide a with the diterpene C-20 alcohol phytol to give chlorophyll a. Although for many years it was thought that chlorophyllide was directly esterified with phytol, it has been demonstrated that chlorophyllide a is first esterified with geranylgeraniol which after three successive hydrogenations is transformed into phytol.

Chlorophyll b, the other chlorophyll present in all green plant tissues, is formed from chlorophyllide b by esterification, as with chlorophyllide a, with geranylgeraniol and followed by three successive hydrogenations to give phytol. Chlorophyllide b is formed from chlorophyllide a by oxidation of the C-7 methyl group to an aldehyde.

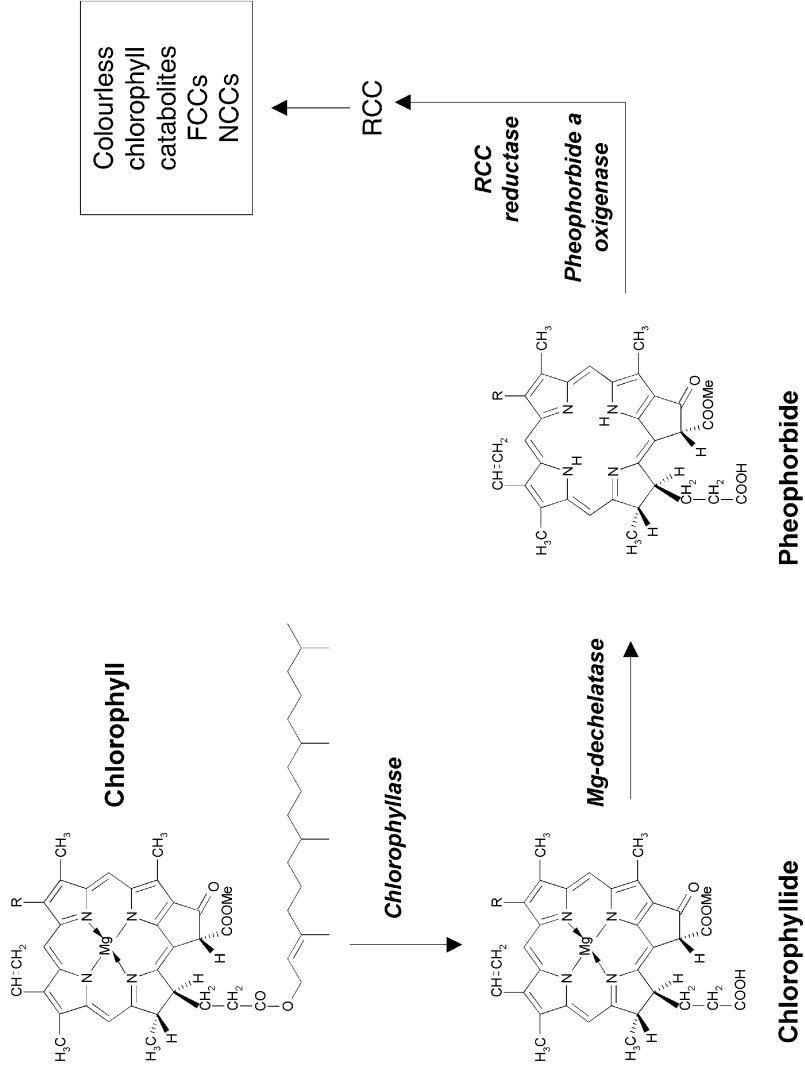
### 10.5.2 Chlorophyll catabolism

Chlorophylls are naturally decomposed during their participation in the photosynthesis process, but a powerful turnover process compensated the decomposition. However, during fruit ripening and leaf senescence chlorophyll catabolism takes place. About 1.2 billion tons of chlorophyll is estimated to be degraded globally each year (Hendry *et al.*, 1987). Biochemical understanding of this natural phenomenon has remained unknown for a long time but in the last decade the mechanism of chlorophyll breakdown during leaf senescence and fruit ripening has gradually been elucidated, although it has always fascinated the scientists (Matile *et al.*, 1997; for a review see Hörtensteiner 1999). During fruit ripening the chlorophylls usually disappear following chloroplast degeneration to gerontoplast. In the green tissues of most plants (leaves) many of the chloroplasts disintegrate but some remain to mask the yellow carotenoid colour. However, in ripened fruits chloroplasts degenerate into chromoplast, a process that is usually accompanied by a massive *de novo* biosynthesis of carotenoids.

The total chlorophyll content and the ratio Chl a/b of fruit varies depending on the plant genus, species and cultivars, as well as the environmental conditions (including agronomic practices) and developing stage (Gross, 1987). Changes in the ratio Chl a/b have been followed in many fruits (apple, peach, orange, olive, pepper, etc.) during fruit ripening and in most fruits the ratio decreased but in some others an increase has been noted, which suggest that in those cases chlorophyll b was more rapidly destroyed than chlorophyll a. In other cases, such as in 'Shamouti' orange, the ratio Chl a/b remained almost constant for a long period although the total chlorophyll content decreased, indicating an equal disappearance of both chlorophylls. Differences in the destruction rates of chlorophyll a and b have been related to preferential action of some chlorophyll degrading enzymes (Mínguez-Mosquera and Gallardo-Guerrero, 1995, 1996).

Brown *et al.* (1991) classified the complicated chlorophyll breakdown pathway into two reaction types. Type I involves the loss of phytol, Mg, and modifications of the side chains of the isocyclic ring. On the other hand Type II reactions include oxidative cleavage (bleaching) of the tetrapyrrole macrocycle in a rapid process that involves molecular oxygen and light. Later it was established that in general terms the Type I chlorophyll breakdown pathway consists of three main steps involving three different enzymes, namely chlorophyllase, Mg-dechelataase, and pheophorbide *a* oxygenase (Vicentini *et al.* 1995). Figure 10.3 shows a simplified scheme of chlorophyll degradation. The first step is mediated by chlorophyllase, which catalyses hydrolysis of chlorophylls to chlorophyllide and phytol. Chlorophyllase or chlorophyll-chlorophyllide hydrolase (EC 3.1.1.14) is an intrinsic membrane-bound enzyme located in photosynthetic membrane systems of higher plants and algae (Moll *et al.* 1978; Khalyfa *et al.* 1993). Chlorophyllase exhibits a high specificity towards the substrate, and was also found to be stereospecific, the carboxymethyl in the C13<sup>2</sup> chiral group playing a very important role in the interaction between substrate and enzyme (Fiedor *et al.* 1992). Genes for chlorophyllase isozymes have been cloned recently (Jacob-Wilk *et al.* 1999; Tsuchiya *et al.* 1999), which will help to elucidate the physiological roles of this and other related chlorophyll catabolism enzymes. Mg-dechelataase is responsible for the removal of Mg ions to produce the Mg-free derivatives, pheophytins and pheophorbides, from chlorophylls and chlorophyllide respectively. Both chlorophyllase and Mg-dechelataase are constitutive chloroplast membrane enzymes that are present in a latent state, that is, not acting on their substrates in normal conditions. Indeed, chlorophyllase has been located in the inner membrane of the chloroplast envelope where there are no chlorophylls to act on (Matile *et al.* 1997; Fang *et al.* 1998), and enzyme action exists only when the chloroplast structure is disorganised during ripening, senescence or ageing. Pheophorbide *a* oxygenase is activated by the ripening and senescent process, oxidising pheophorbide *a* to uncoloured primary fluorescent products (pFCCs), its activity being mediated by molecular oxygen and reduced ferredoxine (Matile and Schellenberg 1996). Pheophorbide *a* oxygenase catalyses the oxygenolytic opening of the porphyrin macrocycle and represents the core piece of the chlorophyll catabolic pathway, being responsible for the loss of the green colour (degreening process – see Section 10.8.2) and yielding colourless products.

Fluorescent (FCCs) and non-fluorescent chlorophyll catabolites (NCCs) have been isolated and identified in several plant species, such as CaFCC in *Capsicum annuum* (Moser and Matile 1997), HvFCC in *Hordeum vulgare* (Kräutler *et al.* 1992), and BnFCC in *Brassica napus* (Mühlecker *et al.* 1993). All of these catabolites have a common open tetrapyrrole structure (which explains their different chromophore characteristics with respect to chlorophylls) as a result of the action of the enzyme pheophorbide oxygenase on the methene bridge linking rings A and B (Rodoni *et al.* 1997). The production of pFCCs from pheophorbide *a* requires the combined action of pheophorbide *a* oxygenase, producing a red catabolite (RCC), and RCC reductase that reduces



**Fig. 10.3** Simplified scheme of the chlorophyll breakdown pathway.

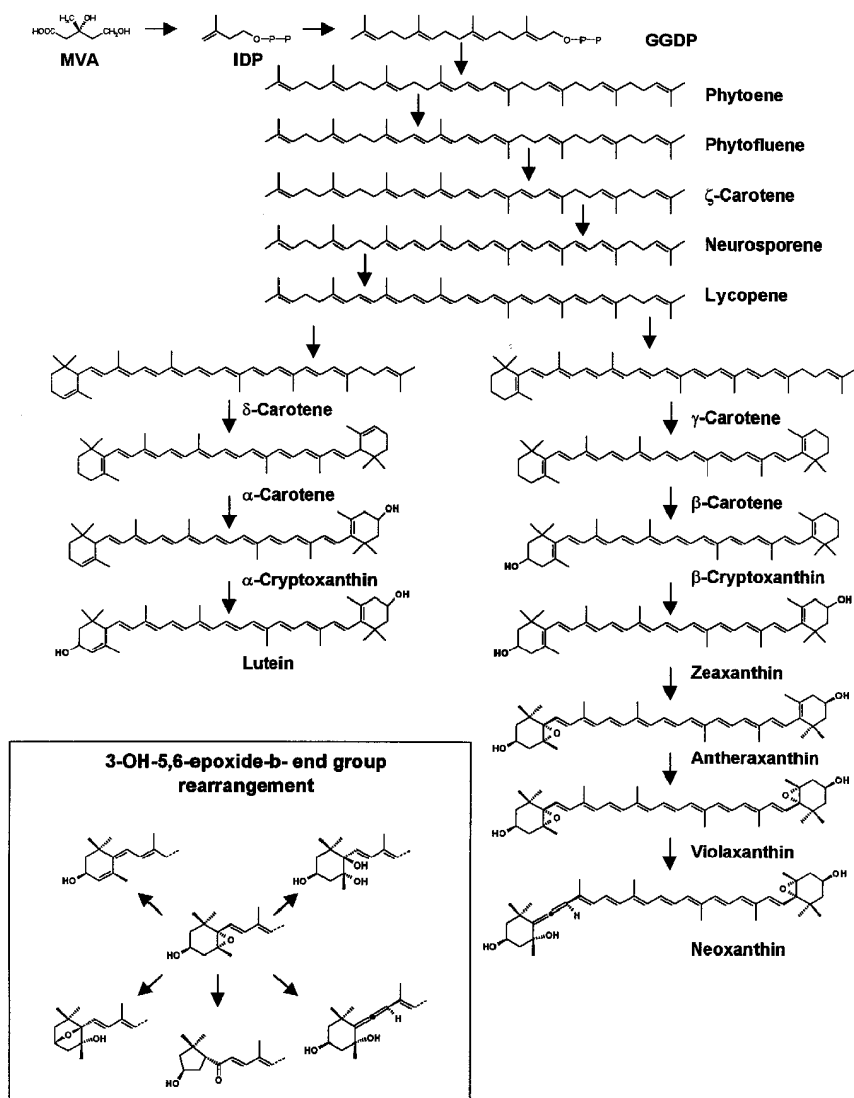
RCC to pFCCs. The pFCCs are converted to other non-fluorescent catabolites (NCCs), exported to the cytosol and stored in the central vacuoles of senescent cells, allowing detoxification of these potent photosensitisers to prevent cell damage. In the case of chlorophyll b, it has been suggested that it enters the degradation pathway after being converted to chlorophyllide b by chlorophyllase. Chlorophyllide b is then converted to chlorophyllide a by the action of chlorophyll b reductase. In addition to this general chlorophyll breakdown pathway, a number of other enzyme systems have been suggested to be implicated in an oxidative bleaching of chlorophylls: peroxidase, lipoxygenase, chlorophyll oxidase and polyphenol oxidase (Martinoia *et al.* 1982).

## 10.6 Carotenoids

### 10.6.1 Carotenoid biosynthesis

The carotenoids are isoprenoid compounds and are biosynthesised by a branch of the great isoprenoid pathway. The entire biosynthesis takes place in the chloroplast (in green tissues) or chromoplast (in yellow to red tissues) encoded by nucleous genes. In carotenoids, the isoprenoid chain is built up from mevalonic acid (MVA) by prenyl transferases to the C20 level, as geranylgeranyl diphosphate, and two molecules of this are joined tail-to-tail to give 15-*cis* phytoene as the first product with the C40 carotenoid skeleton, which is catalysed by the phytoene synthase (PSY). Phytoene is colourless but undergoes a series of desaturation reactions, each of which creates a new double bond and extends the chromophore by two conjugated double bonds. The end product is lycopene, produced via the successive intermediate phytofluene,  $\zeta$ -carotene and neurosporene by the combined action of phytoene desaturase (PDS) and  $\zeta$ -carotene desaturase (ZDS) (Fig. 10.4). The light absorption maximum shifts progressively to longer wavelengths as the chromophore is extended and lycopene, with 11 conjugated double bonds, absorbs maximally ( $A_{\max}$ ) at 470–500 nm and is strongly coloured orange-red. The phytoene in higher plants appears to be formed as the 15Z isomer, though lycopene and the other coloured carotenoids are generally in the all-E form. Isomerisation from Z to E must therefore take place during the desaturation sequence but the stage at which this occurs has not been established unequivocally.

The lycopene molecule may then undergo cyclisation, the branch point that later gives the great variety of xanthophyll structures, to form six-membered rings at one end or both ends of the molecule, e.g.,  $\beta$ -rings and  $\epsilon$ -rings. This reaction is catalysed by two lycopene cyclases. Lycopene  $\beta$ -cyclase catalyses a two-step reaction that forms one  $\beta$ -ionone ring at each end of the lycopene molecule to give  $\beta,\beta$ -carotene. Lycopene  $\epsilon$ -cyclase creates only one ring to produce  $\delta$ -carotene from lycopene or  $\beta,\epsilon$ -carotene from  $\gamma$ -carotene (with only one  $\beta$ -ring). The introduction of oxygen functions and other structural modifications of end groups, including esterification, then follow as the final



**Fig. 10.4** Biosynthetic pathway for the main carotenoids found in fruits and vegetables.

stages of biosynthesis. Thus zeaxanthin and lutein are formed by the introduction of two hydroxy groups at C-3 and C-3' of  $\beta,\beta$ -carotene and  $\beta,\epsilon$ -carotene, respectively by the action of hydroxylases. Following hydroxylation, an epoxide group can be introduced at positions 5 and 6 of the 3-hydroxy- $\beta$  ring. In this way zeaxanthin is converted into violaxanthin via antheraxanthin by introducing respectively two and one 5,6 epoxide groups. Schemes have been proposed for the formation of a variety of other end groups by rearrangement of a 3-hydroxy-5,6-epoxy- $\beta$  ring end group (Fig. 10.4) (Britton and Hornero-

Méndez 1997). In the case of hydroxycarotenoids, it is common in fruits (red pepper, lemon peel, etc.) for it to occur naturally as esters with different fatty acids (Mínguez-Mosquera and Hornero-Méndez 1994b). Is it assumed that fatty acid carotenoid esters are formed conventionally by esterification of the hydroxy groups with the appropriate acyl-CoA, but the biochemistry of the process has not been studied (Britton 1998).

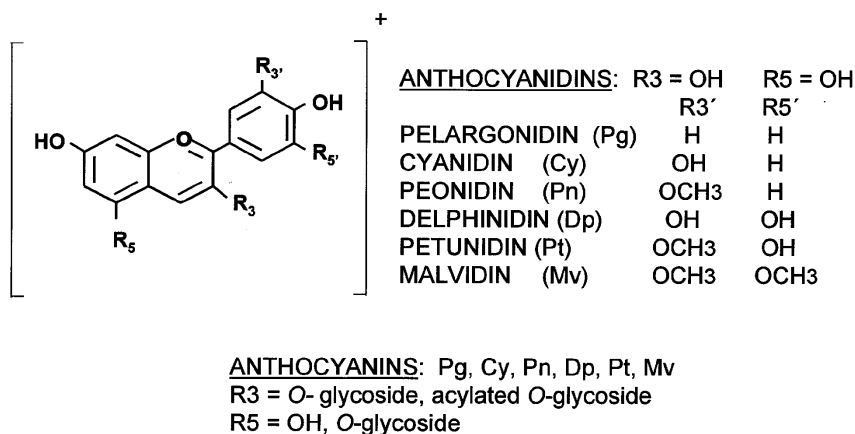
Because of the number of combinations of reactions that are possible in the two ends of the molecule, the conventional pathways that can be constructed can look very complicated. However, the picture is greatly simplified when considered in terms of sequences of reactions that can occur in one end of the molecule or the other. Thus the formation of zeaxanthin from lycopene involves only two reactions, namely  $\beta$ -cyclisation and hydroxylation, at each end group. For instance, the exotic-looking cyclopentanone end group of capsanthin and capsorubin requires only two additional reactions, namely an epoxydation and a rearrangement. Each reaction, whether it occurs in only one or in both end groups, is catalysed by a particular enzyme (Hirschberg 2001).

## 10.7 Anthocyanins

### 10.7.1 Anthocyanins biosynthesis

The anthocyanin biosynthetic pathway has been discussed, established and reviewed by several authors (Dixon and Paiva 1995, Forkmann 1991, Holton and Cornish 1995; Mol *et al.* 1989). The precursors of the synthesis of anthocyanins are malonyl-CoA (coming from 3 acetyl-CoA) and p-coumaroyl-CoA phenylalanine. The stepwise condensation of three acetate units from malonyl-CoA with p-coumaroyl-CoA to yield tetrahydroxychalcone is catalysed by chalcone synthase (CHS) enzyme. Thereafter chalcone isomerase (CHI) catalyses the isomerisation of the tetrahydroxychalcone (yellow coloured) to naringenin (colourless), that is transformed into dihydrokaempferol (DHK) by the enzyme flavanone 3-hydroxylase (F3H). Subsequently DHK can be hydroxylated by flavonoid 3'-hydroxylase (F3'H) to produce dihydroquercetin (DHQ) or by flavonoid 3'-5'-hydroxylase (F3'5'H) to produce dihydromyricetin (DHM). DHQ could be also transformed to DHM by the action of F3'5'H. Later the colourless dihydroflavonols (DHK, DHQ and DHM) are transformed into anthocyanins by the action of three specific enzymes. The first step in this conversion is the enzymatic transformation of dihydroflavonols to flavan-3,4-cis-diols (leucoanthocyanidins) by dihydroflavonol 4-reductase (DFR). Subsequent oxidation, dehydration and glycosylation of the different leucoanthocyanidins led to the corresponding pelargonidin (brick-red), cyanidin (red), and delphinidins (blue) anthocyanins pigments. Finally, glycosylation, methylation and acylation in several species could modify anthocyanidin 3-glucosides (Holton and Cornish, 1995). Figure 10.5 shows structures for anthocyanidins and anthocyanins pigments.





**Fig. 10.5** Structures for anthocyanidins and anthocyanins pigments.

## 10.8 Postharvest changes in fruit pigment composition

Biochemical changes during the ripening of fruit and vegetables include several metabolic processes including both biosynthetic (anabolism) as those causing degradation of natural fruit components (catabolism). Among the anabolism processes could be included the biosynthesis of ethylene (considered the main plant hormone), organic volatile substances responsible for aroma, several aminoacids, proteins and enzymes, ribonucleic acid, and some pigments like carotenoids and anthocyanins. Among the most important catabolism processes, hydrolysis of starch, pectics substances and tannins, reduction of organic acids, and degradation of chlorophyll can be included. For many fruit and vegetables, loss of chlorophyll, accompanied by disintegration of chloroplast membranes, is considered the first step in ripening (Kader 1992).

Most of these naturally occurring changes associated with ripening, led to a better acceptability of fruit and vegetables by consumers, in particular changes related to firmness and texture, flavour and colour. For most fruit and vegetables, changes during ripening of weight, sugars, acidity and chlorophyll content are common. However, changes in respiratory activity ( $O_2$  consumption and  $CO_2$  emission), flavour and carotenoids content depend on the kind of product. As previously cited (see Section 10.3) chlorophyll loss could occur without an increase in carotenoids (e.g., ripening of banana), or the increase in carotenoids could occur without a loss of chlorophyll (e.g., in some hybrids of tomato and peppers) although in most fruit and vegetables, degradation of chlorophyll and biosynthesis of carotenoids or anthocyanins takes place simultaneously.

### 10.8.1 Changes in colour according to fruit respiratory activity

The respiration rate during ripening of fruit and vegetables is not uniform and changes depending on plant species. Respiration rate changes can distinguish

two kinds of fruit. One shows how from the beginning of ripening the respiration rate progressively decreases until a minimum value is reached, and then suddenly increases to a maximum (climacteric pick). This maximum generally coincides with the fully ripe stage, when sensorial quality attributes (including colour) are at the optimum level for consumption. The intensity and duration of the climacteric depend on specie. After this the respiration rate progressively decreases, corresponding to the degree of fruit senescence, until the organ is physiologically dead. These kinds of fruit and vegetables, named climacteric, include pome fruits, stone fruits (except cherry), most of the tropical fruits (banana, plantain, avocado, guava, cherimoya, etc.), tomato, melon, etc. Other kinds of fruits and vegetables follow this pattern showing a progressive decrement in the respiration rate throughout ripening until senescence and the death of the fruit. These fruits named non-climacteric, include, for example, citrus, cherry, pomegranate, grape, berries and most vegetables (Artés *et al.* 2000; Kader 1992).

### 10.8.2 Climacteric and non-climacteric fruit

During climacteric increase maximum degradation of chlorophyll occurs, although generally some small quantities of chlorophyll are always present in the internal tissues. It has been observed in apple and pear that degradation of chlorophyll could be mainly due to hydrolytic activity of chlorophyllase enzymes that transform chlorophyll into phytol and porphyrin and the resultant chlorophyllide has no effect on colour changes. However, in tomato, this effect was not observed and disintegration of chloroplast membranes occurs before the loss of green colour (Pantastico 1979).

During ripening of non-climacteric fruits like *citrus*, the process of colour change is named degreening and the natural loss of chlorophylls, accumulated into the chromoplasts of the epidermis (flavedo) and vesicles, and the concomitant manifestation and new biosynthesis of carotenoids, generally occurs very slowly (Eaks 1977).

### 10.8.3 Influence of postharvest treatments

In their natural location within the chloroplast, the chlorophylls are compounds with moderate stability. However, when this organelle is disorganised, damaged and loses its physiological conditions, chlorophylls become extremely labile, susceptible to a wide range of structural modifications by factors such as temperature, acid-basic conditions, enzyme action, molecular oxygen, and light, and as a result of almost any postharvest and processing operation, leading to the formation of a variety of chlorophyll derivatives. Figure 10.6 shows the major transformations of the chlorophyll molecule under different physico-chemical conditions and/or enzyme actions. One of the main transformations is the formation of pheophytin by interchange of the magnesium ion by protons from the medium. Another common transformation is the formation of chlorophyllide

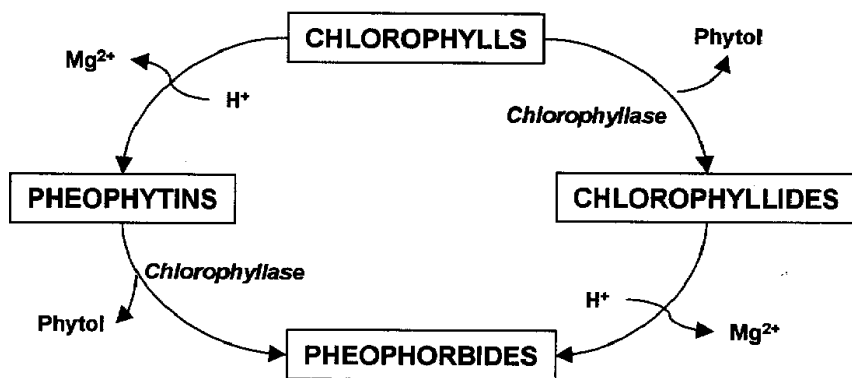


Fig. 10.6 Main modifications of the chlorophyll molecule.

after the hydrolysis of the phytol ester by the action of chlorophyllase enzymes. Both chlorophyllide and pheophytin may now undergo magnesium loss or phytol hydrolysis respectively to form pheophorbides. The substitution of Mg by protons produces a drastic colour change, from green to grey, in the resulting pheophytin, whereas the hydrolysis of the phytol ester produces chlorophyll derivatives with higher polarity (chlorophyllide) (Mínguez-Mosquera *et al.* 1997).

The acceleration of ripening in climacteric fruit or the degreening process in non-climacteric ones is interesting because it allows anticipation of the selling period, when prices are higher, as well as improving fruit quality because of more even uniformity of external skin colour. Ripening and degreening could be accelerated by increasing temperature and ethylene action. The usual range of temperature for acceleration of colour changes depends on the kind of product, specie and cultivar, expected duration of process and consumer demand on external fruit colour. Optimum temperature for carotenoids biosynthesis depends on the type of carotenoid and product and commonly is in the range of 15 to 25°C. As an example, for lycopene in tomato the range is from 16 to 21 °C and at 30 °C or more it is inhibited. However, biosynthesis of lycopene in watermelon still continues at 37 °C and in 'Redblush' grapefruit at temperatures higher than 30 °C (Wheaton and Stewart 1973). Commonly, degreening of citrus fruit is conducted at 1 to 10 ppm of ethylene in the atmosphere around the fruits at temperatures between 18 and 30 °C and 90–95% relative humidity (Artés *et al.* 1978 and 1994; Cuquerella 1997; Davies and Albrigo 1994; Eaks 1977). For Spanish citrus fruits the recommended degreening temperatures are 18 to 21 °C for mandarins and 20 to 22 °C for oranges (Artés *et al.* 1994, 2000; Cuquerella 1997; Orihuel 1986) and 21 to 23 °C for lemons and grapefruits (Artés *et al.* 1978, 1983, 1997, 2000).

A certain activity of methyl jasmonate at about 8 ppm concentration for the acceleration of ripening in apples and degreening in oranges, due to stimulation of ethylene biosynthesis, has been reported (Olías *et al.* 1990). However, these results seems to be inconsistent at least in orange (Artés *et al.* 1994, 2000).

## **10.9 Fruit colour, pigment composition and quality**

The colour of fruits is considered one of the most important quality attributes. In fact consumer acceptance is firstly determined by colour and secondly by other attributes such as flavour, texture, etc. A natural appearance of a fruit is always positively valued, whereas caution is exercised when a fruit presents an unexpected colour, which is frequently interpreted as deterioration or careless handling of fruits. Many quality control protocols use colour as an attribute to measure the degree of quality of fruits, and foods in general, and therefore to assess the commercial value of the product. In this way colour can be used to define quality (colour as quality). However, as previously mentioned in this chapter, the colour of fruit and vegetables is a direct consequence of their natural pigment composition. Therefore, only after identification and quantification of each individual pigment present in a given kind of fruit it will be possible to characterise the material and provide tools for authentication of the fruit (and derived products) and the detection of alterations or deviations in pigment composition. Eventually it will be possible to assess the 'quality of the colour'. Colour as a quality, and the quality of this colour must be thought of as inseparable (Roca L.-Cepero and Mínguez-Mosquera 2001b).

Enzymatic, microbiological and physico-chemical processes may notably affect the characteristics of fruit, including colour, during the ripening and postharvest period, as well as during transport and storage imposed by market demands. There is, therefore, an increased demand of research projects dealing with and focused on the understanding of the processes involved in the modification of the different fruit components, to allow a better fruit selection, control the postharvest treatments and ensure high and consistent quality levels.

The structure of chlorophylls, carotenoids and anthocyanins present in fruits will be affected to some degree during the development, ripening, and postharvest treatments, with a consequent effect on the colour (quality and quantity) and nutritional value (i.e., carotenoids are a provitamin A source) of the final product.

## **10.10 Physico-chemical and enzymatic factors affecting fruit stability**

Factors such as light, pH, relative humidity, gas composition and enzymatic systems are involved in the colour deterioration of fruits and vegetables. As an example, it has been found that ethylene produced during cutting in minimally fresh processed spinach notably accelerates the loss of chlorophylls, and damage is proportional to the ethylene level reached (Abe and Watada 1991).

In most cases an optimisation of harvesting and postharvest treatments would be needed for each kind of fruit and variety, and this can only be carried out after a full characterisation of the pigment composition. A deep knowledge of the pigment metabolism, including biosynthetic capacity, implicated enzymes and

identification of breakdown catabolites, is necessary to decide the optimum fruit harvest date. The pigment composition will make it possible to define the inherent pigment present in each kind of fruit and to distinguish between cultivars on the basis of pigment profile, ratios and absence or presence of exclusive pigments.

### 10.11 Measuring colour and pigment composition

With the wide availability and development of sophisticated chromatographic (HPLC, TLC) and spectrophotometric (UV-vis, colourimetry, etc.) analytical techniques, the analysis of pigment composition has now become routine. In the last 40 years many advances have been made to establish destructive and non-destructive methods for the estimation not only of pigment composition but also colour quality indices. One interesting example is ripe red pepper fruits, which owe their intense red colour to carotenoid pigments (mainly the reddish capsanthin and capsorubin) synthesised massively during ripening. Colouring power is the main characteristic determining the commercial value of paprika, and is directly related to the total content in carotenoid pigments (Mínguez-Mosquera *et al.* 1992b). Apart from total carotenoid content, a higher proportion of red (R) pigments than yellow (Y) ones is considered positive, making the R/Y ratio a direct quality criterion (Hornero-Méndez and Mínguez-Mosquera 2001; Mínguez-Mosquera *et al.* 1984).

Both developing industry and potential buyers need analytical methods that are simple, quick, and readily available, enabling a rapid evaluation of product quality. The most important of the usual methods is that of the American Spice Trade Association, the ASTA-20.1 (ASTA, 1986) for extractable colour, and that of chromatic attributes ( $L^*$ ,  $a^*$ ,  $b^*$ ) proposed by the Commission Internationale de l'Eclairage (CIE 1986). However, such methods furnish measurements of colour, though not necessarily that from carotenoids. Other more reliable methods include chromatographic separation prior to quantification. Some methods employ TLC (Mínguez-Mosquera *et al.* 1984; Vinkler and Kiszal-Richter 1972) and others HPLC (Mínguez-Mosquera *et al.* 1991b; Mínguez-Mosquera *et al.* 1992a; Mínguez-Mosquera and Hornero-Méndez 1993, Müller 1997). Such methods yield information about individualised pigment composition and total carotenoid content of the sample, and allow the pigment ratios to be calculated.

Rapid spectrophotometric methods have recently been developed to estimate the concentration of the R and Y isochromic fractions and the total carotenoid content in oleoresins that could be used as a quality index (Hornero-Méndez and Mínguez-Mosquera 2001, Mínguez-Mosquera and Pérez-Gálvez 1998). The methods define independent equations for the R and Y fractions and allow determining the ratio R/Y and the total carotenoid content.

In the case of olive fruit the ratio of total chlorophylls to total carotenoid content (Chl/Car) has also been used to characterise cultivars and establish the

ripening stage of the fruit. Normal values for Chl/Car in olive fruits are 3–3.5, while in the virgin olive oil extracted from these fruits the ratio takes a value of 1 due to the preferential extraction of carotenoids versus chlorophylls (Roca and Mínguez-Mosquera 2001b). Relative composition (in percentage) for some carotenoids such as violaxanthin, lutein and  $\beta$ -carotene have also been used to characterise and authenticate single variety virgin olive oils (Gandul-Rojas *et al.* 2000). Recently the HPLC carotenoid profiles have been applied for the authentication and determination of geographical origin of 'Valencia' orange juice and for the adulteration of orange juice with paprika extract and tangerine juice (Mouly *et al.* 1999).

Other colour indices are used to measure the maturity stage of fruits. Red pepper is again a good example of this. Apart from the pigments ratios (such as R/Y) the esterification of xanthophyll has been measured during the ripening of several fruits (orange, red pepper, apple, peach, etc.). Commonly, a gradual accumulation of total xanthophylls is accompanied by a concomitant increase in xanthophyll esters, in some cases up to 60–75% of the total xanthophylls. Today, there is an essential need for a uniform fruit maturity stage, to ensure product quality and to take full advantage of the biosynthetic capacity of the fruits. This is crucial when the red pepper fruits are used for paprika production (Hornero-Méndez *et al.* 2000, Mínguez-Mosquera and Hornero-Méndez 1994a, Mínguez-Mosquera *et al.* 2000). The xanthophyll esterification process seems to be intimately linked with and controlled by the ripening process. The balance reached between the esterification of xanthophyll fractions (free, partially esterified, and totally esterified), with mean values of 25, 30 and 45% respectively, seems to be well kept between varieties, and thus could be used as a maturity index for pepper fruit (Hornero-Méndez and Mínguez-Mosquera 2000; Mínguez-Mosquera and Hornero-Méndez 1994b). The use of this index would ensure uniformity at the ripening stage of fruit and take maximum advantage of their carotenogenic capacity, with a direct impact on the quality of the processed products, paprika and oleoresins.

The anthocyanin profile usually changes during fruit ripening and maturation. It has been extensively studied in the rich anthocyanins content of pomegranate fruit. In fact, the diglucoside derivatives are the predominant pigments during the early ripening stages (cyanidin 3,5-diglucoside being the main pigment) while the monoglucoside derivatives (mainly cyanidin 3-glucoside) prevail in the later stages (Artés *et al.* 2000 a,b; Gil *et al.* 1995a,b, 1996c; Harbone 1967). Commonly, there is an increase in juice pigmentation throughout the different maturity stages and as an example, in 'Mollar' cv, the total anthocyanin content varies from 5 mg/l in the 26th week after flowering to 389 mg/l in the 32nd week when fully ripe (Gil *et al.* 1996c). Red colour of pomegranate juice has been reported to continue increasing in intensity throughout storage at 2 to 5 °C in 'Wonderful' (Ben-Arie *et al.* 1984) as well as in 'Mollar' cultivar (Gil *et al.* 1995c) or at least it was stabilised on the same cultivar (Gil *et al.* 1996c; Kader *et al.* 1984).

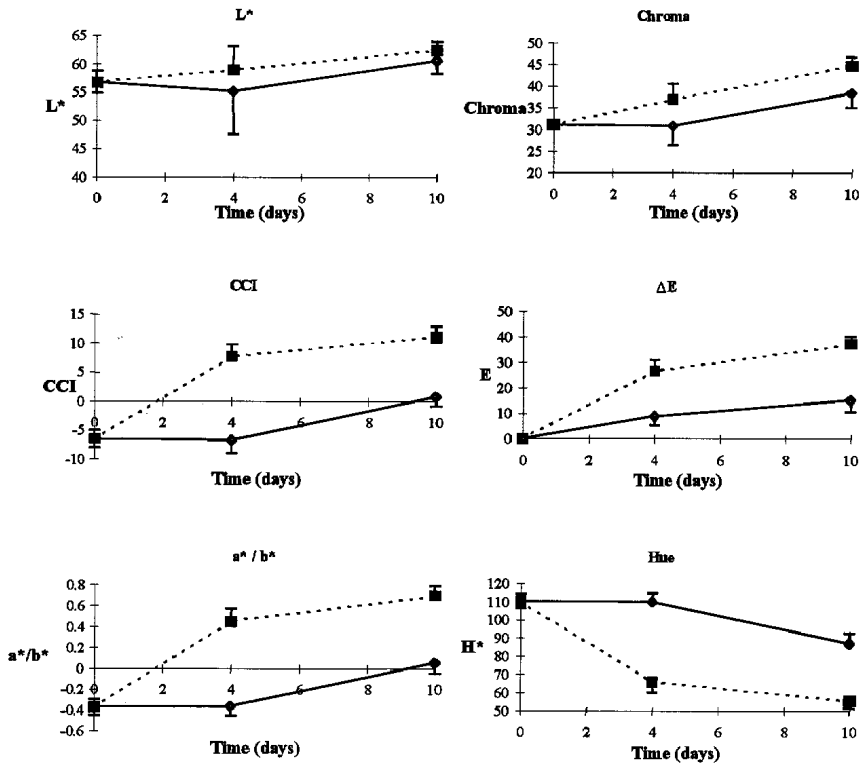
Changes in colour are usually analysed by mean of different indices in order to avoid difficulties in working with the three numerical data corresponding to

the  $L^*$ ,  $a^*$  and  $b^*$  chromatics colour coordinates of the CIELAB colour measurement system (CIE, 1986). In addition, these three values taken independently do not provide information about tone and intensity of measured colour. Consequently some colour indices have been developed as described below.

The ratio  $a^*/b^*$ , which was a successor to the use of the Hunter  $a/b$  ratio, is highly functional in emphasising red/yellow changes, and has been successfully applied to determine colour changes during degreening of lemons (Barmore and Wheaton 1976) and during postharvest ripening of tomato (Artés and Escriche 1994). However, it is in fact a distorting index because of the tangential nature of the values. The metric Chroma  $[(a^{*2} + b^{*2})^{1/2}]$  index, also named the saturation index, represents the distance from the coordinate's origin to the determined colour point (Shewfelt *et al.* 1988). The Hue angle  $[\arctg(b^*/a^*)]$  index represents the tone of colour that commonly decreases during degreening ( $90^\circ$  represents a yellow colour, higher values indicate green and lower orange) (Gnanasekharan *et al.* 1992; Little 1975). As a specific colour index for citrus fruit Jiménez *et al.* 1981 proposed the Citrus Colour Index ( $CCI = 1000 a^*/L^*b^*$ ), that usually shows a high correlation to visual appreciation of the flavedo colour. Finally, the index named Total Increment of Colour or Total Colour Difference,  $\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2}$  has been proposed by Mackinney and Little (1962) and used by Gnanasekharan *et al.* (1992) for determining colour changes in vegetables (the parameters without subindices refer to a certain moment during the process of colour change and those with subindex 0 to the initial ones).

These indices have been applied to represent changes in colour throughout the continuous degreening system of 'Eureka' lemon, 'Henderson' grapefruit and 'Newhall' orange with 5 ppm of ethylene for four days at  $22^\circ\text{C}$  and 90–95% RH, followed by storage at  $20^\circ\text{C}$  and 70–75% RH for six or seven days to simulate a maximum marketing period. As expected, quality attributes such as titratable acidity, pH, total soluble solids content and maturity index did not show significant changes when comparing fruits treated with ethylene and control ones, according to the normal behaviour of non-climacteric fruits, corresponding to *citrus*. However, significant colour differences in the flavedo were observed in orange, lemon and grapefruit at the end of the degreening process and after a suitable marketing period. Commonly the Hue, Chroma and  $a^*/b^*$  colour indices were the most sensitive to reflecting the changes in external colour. Orange is commonly the fruit most easily degreened and grapefruit the most difficult one.

Generally, no important losses were observed as a result either of fungal attacks or of physiological disorders during citrus degreening for four days at  $22^\circ\text{C}$ , 90–95% RH and 5 ppm ethylene in continuous systems. The weight losses at the end of the degreening period did not commonly exceed 1%. However after a marketing period of six to seven days at  $20^\circ\text{C}$  and 70–75% RH, in different laboratory experiments, mean weight losses reached about 3.5% in grapefruit, 4.5% in orange and 5.5% in lemon (Artés *et al.* 1978, 1994, 1997, 2000c).



**Fig. 10.7** Changes in  $L^*$  parameter and colour indices (Chroma, CCI,  $\Delta E$ ,  $a^*/b^*$ , and Hue) on the flavour of 'Newhall' orange during degreening at 22°C and 95% RH on untreated (control) and continuous 5 ppm  $C_2H_4$  treated fruit, and after subsequent six days at 20°C and 70–75% RH (retail sale and distribution simulated period).

From our results during degreening of 'Newhall' orange, the treatment with methyl jasmonate at concentrations of 3 mg JAMe/L in the degreening room did not prove to be effective for accelerating degradation of chlorophyll pigments.

Figure 10.7 shows an example of changes in some colour indices during degreening of oranges. On the other hand, changes in chemical quality attributes during degreening of grapefruit are reported in Table 10.3.

## 10.12 Future trends

Future research should be focused on genotypic variation in pigments content of fruit and vegetables. In fact, presently there are increasing opportunities to develop genotypes by using classical breeding and molecular-based methods for varietal development that have enhanced sensorial quality including higher carotenoids and polyphenols content and reduced browning potential. However,



**Table 10.3** Changes in some chemical parameters during degreening of 'Río Red' grapefruit at 22°C, 95% RH on untreated (control) and continuous 5 ppm C<sub>2</sub>H<sub>4</sub> treated fruit, and subsequent shelf life of six days at 20°C and 65–70% RH

Days	Soluble solids content (°Brix)		pH		Titratable acidity (% citric acid)		Maturity index SSC/TA)	
	C <sup>z</sup>	T <sup>y</sup>	C	T	C	T	C	T
0 <sup>x</sup>	9.7±0.3 <sup>w</sup>	9.7±0.3	3.16±0.05	3.16±0.05	1.38±0.08	1.38±0.08	7.03±0.59	7.03±0.59
4	10±0.3	10.3±0.2	3.40±0.04	3.30±0.03	1.46±0.11	1.37±0.04	6.85±0.65	7.52±0.31
10	9.5±0.4	9.8±0.2	3.32±0.12	3.58±0.06	1.36±0.16	1.37±0.05	6.99±1.02	7.15±0.43

z: Control fruits      y: Treated fruits with 5 ppm C<sub>2</sub>H<sub>4</sub>.

x: 0 = At harvest; 4 = After 4 days of degreening; 10 = After 6 days of shelf life at 20°C and 65–70% RH.

w: Figures are means (*n* = 8) ± standard deviation.

these goals may be contradictory, such as lowering phenolic content and activities of phenylalanine ammoniolyase and/or PPO to reduce browning potential versus increasing polyphenols as pigments as well as antioxidants with positive effects on human health. On the other hand, additional research is needed to optimise preparation and subsequent handling procedures for maintaining quality of each plant product. Particularly, temperature, relative humidity and gas composition during storage and transport are the most critical factors in preserving the quality of these fresh and processed fruit and vegetable products (Kader 2001).

Research needs include studies about the relationship between antioxidant capacity and carotenoid, chlorophylls and anthocyanins structures as well as that of their derivatives found in fresh and processed fruits and vegetables. Also the stability and biodisponibility of pigments depending on their nature and interaction with the lipidic environment must be elucidated.

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# 11

## Improving natural pigments by genetic modification of crop plants

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### 11.1 Introduction

Genetic engineering of plants is a new approach developed during the last two decades that allows the modification of the expression of genes after the introduction of an external sequence of DNA in the plant genome. The inserted DNA either expresses a novel activity, reinforces an existing one or may suppress the endogenous expression of a gene in the transformed plant. There are two basic requirements for the genetic manipulation of plants, the introduction of a selected DNA sequence and a protocol for the transformation and regeneration of a particular plant species. In order to select the DNA sequence, some basic information about its role, biosynthesis and regulation is required. Similarly, an efficient genetic transformation system has to be developed for a given crop species. Lack of information about these two aspects set the limits for the application of the plant's genetic engineering tools.

Colour in fruits, as in flowers or other organs of a plant, is determined by many compounds that absorb visible light. Among these there are three general groups known as flavonoids, carotenoids and betalains, whose contribution to fruit colour is considerable (Godoy-Hernández and Lozoya-Gloria 1999). Significant advances have been made in the study of biosynthetic pathways, several of the genes involved have been isolated in a number of model plants and their regulation is known. Genetic modification of plants to improve fruit colour is still at a preliminary level compared to other subjects of plant biotechnology like pathogen defence or herbicide tolerance. The research work published on the modification of these compounds is presented in this chapter.

## 11.2 The genetic modification of crop plants

Traditionally, the development of crops with highly appreciated traits has been achieved by selective cross-breeding techniques. A sexual cross between two parental lines allows the exchange of genetic material that is reflected in the inheritance of the genes and characters among the individuals of the progeny. The choice of the best individuals within this progeny for further sexual crosses allows, when done iteratively, the selection of individuals that gather in their genome the best of the original parental lines. The fundamentals of this procedure are based on Mendel's laws on the inheritance of genetic characters in sexual crosses. In agriculture, such breeding has resulted in the development of cultivated plants with characteristics very different from their ancestral wild species.

The development of recombinant DNA technology has been an important tool for the direct manipulation of plants. The exchange of numerous genes between individuals has been substituted by the insertion of a single, or a few genes in the genome of a receptor plant. The gene introduced, the transgene, gives the name to the transgenic plant, which is also imprecisely named as a genetically modified organism (GMO). The technique is often described as genetic engineering. The introduced gene is translated into a protein which, if involved in a metabolic or physiological step, is going to modify the transgenic plant and, consequently, its phenotype. In the case that constitutes the scope of this chapter the transgene must encode for an enzyme involved in the synthesis of coloured compounds. Its modification in the level of expression in the transgenic plant will eventually change the colour of the organ where the transgene is expressed.

A prerequisite for the application of this technology is the identification and isolation of the gene responsible for the change sought in the transgenic plant. This is currently performed by what is known as DNA recombinant technology. Recent advances in the sequencing of the complete genome of several plant species will constitute a broad source of genes to be used in genetic engineering projects.

The ability to introduce foreign genes into plants, such that they are stably expressed and transmissible from generation to generation, depends on the development of plant transformation systems. This technology consists in the introduction of the selected foreign gene in the receptor plant cells and the development of a protocol for the regeneration of the complete transgenic plant starting from the transformed cells of the receptor plant (Walden and Wingender 1995, Glick and Pasternak 1998). There is no universally applicable method of transformation and regeneration, as tissues from different plant species differ in their response to culture. In general, in dicot plants, such as *Solanaceae*, regeneration of whole plants from explants of different tissues can be achieved at high frequency, while in monocots, plant regeneration frequencies are much lower and are highly dependent on the source of tissue. Thus, highly regenerable tissues are derived from immature and undifferentiated tissues or organ explants.

Currently, a variety of transformation methods have been developed, and this has allowed the transformation of many of the world's most important crop plants.

Plant transformation techniques allow the delivery of the transforming DNA through the cell wall and plasma and nuclear membranes, without compromising the viability of the cell. Gene delivery can be performed either via a biological vector (plant viruses or bacteria) or by non-biological vector-free procedures (chemical methods, microinjection, particle bombardment, etc.) (Birch 1997).

The most commonly used biological vector is *Agrobacterium tumefaciens*, the causal agent of crown gall disease. The genome of *A. tumefaciens*, as in many other bacteria, consists of a chromosome and some autonomous elements known as plasmids (small double-strand circular DNA molecules). *A. tumefaciens* is commonly found in soil and infects many susceptible plant species through sites of mechanical wounding in the root system. The infection process involves the transfer of a portion of plasmid DNA (T-DNA) to the chromosomes of the host plant. Insertion of a selected gene in the T-DNA region of the *Agrobacterium* plasmid using recombinant DNA techniques can be successfully employed to introduce the gene into a plant genome. In order to establish this delivery system for genetic engineering purposes it has been necessary to overcome the inherent limitations of the system while maintaining the advantageous features. Broadly, modified *Agrobacterium* plasmids have been developed in which the genes responsible for disease symptom formation have been removed but retain those necessary for T-DNA excision and integration.

Non-biological transformation systems were developed primarily because *Agrobacterium*-based systems have not been successful in the majority of monocotyledonous species. The technique most developed in recent years is the microprojectile bombardment (particle gun or biolistic technique). Basically, microprojectiles of tungsten or gold are coated with the selected DNA and shot to the target plant tissue by an explosive or electrical discharge, or by pressurised helium. Once the foreign DNA is introduced into the shot cells, it can remain as episomes or it can be integrated into the plant genome by, as yet, unknown mechanisms. If the goal of the transformation is to analyse the function or regulation of a gene, transient expression of the inserted gene may be sufficient. However, for the production of transgenic plants, the introduced DNA must integrate into the genome of the plant cell and the transformed cell must be selected and regenerated into a whole plant. Selective markers present in the introduced DNA allow selection of transformed cells (e.g., genes that confer resistance to antibiotics or herbicides).

Once the selected gene has been introduced into the plant cell genome, it is necessary to regenerate a complete adult plant. In many crops this is the bottleneck for a stable plant transformation. However, many sorts of tissue culture techniques are now available. Cell division can be induced using plant growth regulators (e.g., cytokinins and auxins) to generate callus from several plant tissues or protoplast and changing the concentration of growth regulators,

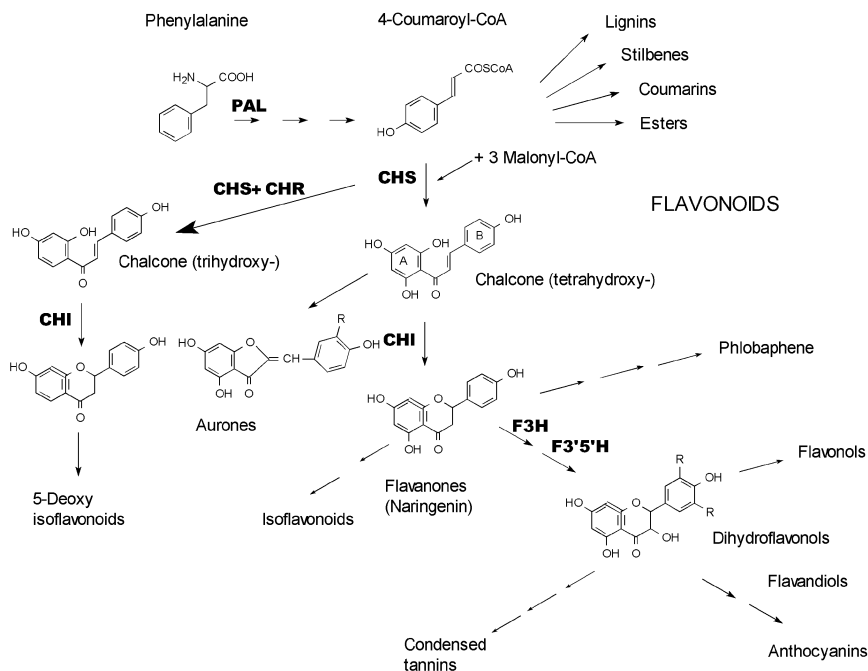
organogenesis can be induced to stimulate shoot and root formation. It is important that other plant characteristics are not modified during the tissue culture regeneration process, this effect is known as somaclonal variation. This can be minimised by shortening the period of tissue culture and using semi-organised tissues instead of protoplasts as targets for transformation. Several transgenic plants are currently being produced with modified steps in metabolic pathways to produce the desired products. Most known cases of transgenic crops have been performed in soybean, maize, and tomato. Those related to changes in plant pigmentation and colour are described in the following sections.

### 11.3 Pigments in fruits

Flavonoids are a large class of plant natural products of low molecular weight. Over 3,000 different flavonoids have been chemically characterised and novel ones are still being reported. Flavonoids are aromatic molecules synthesised from the amino acid phenylalanine and an acetate-derived precursor as malonyl-coenzyme A (Fig. 11.1) (Winkel-Shirley 2001). This reaction is carried out by the enzyme chalcone synthase (CHS) to produce chalcone. The chalcone can subsequently be isomerised by the enzyme chalcone flavone isomerase (CHI) to yield a flavanone. From these intermediates the pathway diverges into several side branches yielding different subclasses of flavonoids, as summarised in Fig. 11.1. These compounds include colourless subgroups such as isoflavonoids, flavones, flavonols and flavandiols, yellow coloured subgroups such as chalcones and aurones, and the coloured anthocyanins, condensed tannins and phlobaphenes pigments which are responsible for most of the orange, scarlet, purple, red, violet and blue colours. Besides the colouration they provide to plants, flavonoids also play important roles as defence and UV-protecting compounds and as signalling molecules in reproduction, pathogenesis and symbiosis (Shirley 1996).

All flavonoids carry a hydroxyl group at the 4' position (ring B), being hydroxylation in other positions controlled by the activities of the corresponding enzymes (Fig. 11.1). Interestingly, the hydroxylation pattern of the B ring of anthocyanins is a major determinant of the colour of these pigments. Whereas phlobaphenes are responsible for the purple colour of maize grains, and the condensed tannins give brown colour to the seeds of different species, anthocyanins, which represent the most conspicuous pigments among the coloured flavonoids, are responsible for the red and purple colourations in the majority of plant species (Fig. 11.2).

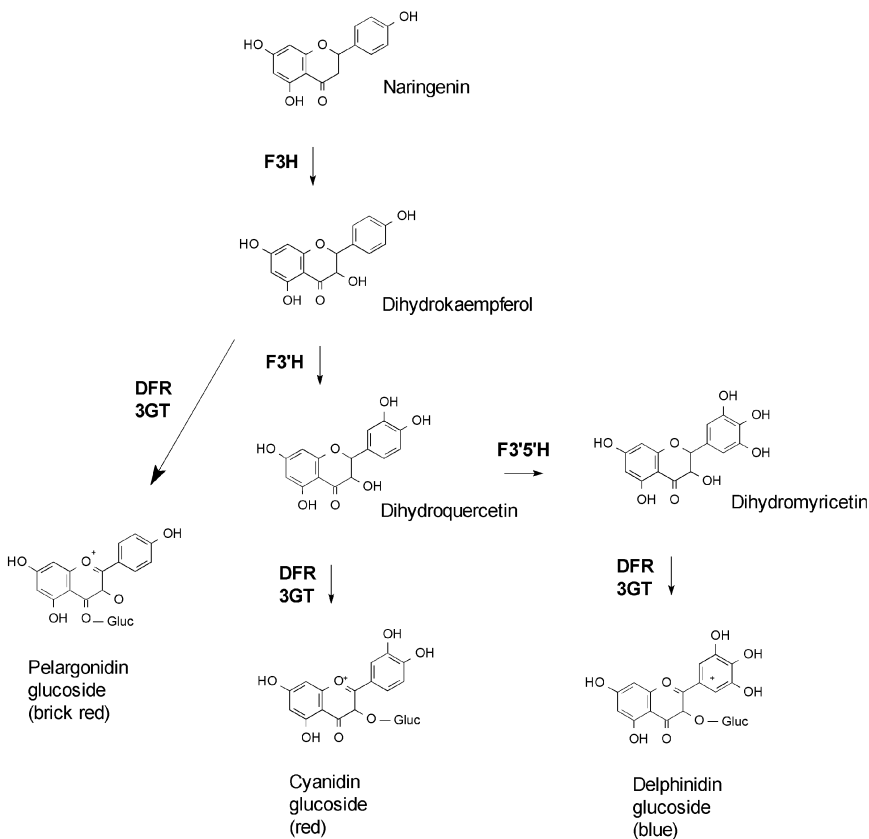
Anthocyanins are widely distributed in nature, occurring in most higher plants and found in all parts of the plants, being most important in flowers and fruits. Analysis of anthocyanins in fruits has revealed that the profile of the anthocyanin content varies among different species, and even within one species. Thus, differentiation of different grape species (species of *Vitis vinifera*) has been possible through the study of the anthocyanin composition. In general,



**Fig. 11.1** Simplified diagram of the flavonoid biosynthetic pathway, starting with the general phenylpropanoid metabolism and leading to the main types of flavonoids. Only a few examples are illustrated of the large variety of flavonoids that arise through modification at different positions (not indicated or shown as R). Enzymes catalysing some key reactions are indicated by the following abbreviations: PAL, phenylalanine ammonia-lyase; CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroflavonol reductase; F3H, flavanone 3-hydroxylase; F3'5'H, flavonoid 3'5'-hydroxylase

it can be said that the anthocyanin concentration for most of the fruits ranges from 0.1 to 1.0% of dry weight (Godoy-Hernández and Lozoya-Gloria 1999).

Carotenoids represent another group of plant pigments that are also found in bacteria, fungi and animals. The colours of many flowers, fruits and vegetables are determined by carotenoids. They also provide colour to certain animals, e.g., some insects, fish and birds. However, plant carotenoids constitute the source of animal carotenoids since they are not able to synthesise them *de novo*. Carotenoids are C40 terpenes produced in the isoprenoid biosynthetic pathway and represent essential components of the photosynthetic membranes in all plants, algae and cyanobacteria (Cunningham and Gantt 1998) (Fig. 11.2). Carotenoids are precursors of vitamin A in animal and human diets and have been shown to be beneficial in reducing coronary heart diseases and cancers. Carotenoids are divided in two groups, carotenes that are either linear or cyclised hydrocarbons and xanthophylls which are oxygenated derivatives. The most prominent chemical feature of carotenoids is the polyene chain, of 3–15



**Fig. 11.2** Diagram corresponding to the biosynthetic pathway of anthocyanins from the intermediate naringenin. Enzymes catalysing the reactions are indicated by the following abbreviations: F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3'5'-hydroxylase; DFR, dihydroflavonol reductase; 3GT, 3-*O*-glucosyl transferase.

conjugated double bonds, which is responsible for the light absorption and, therefore, the colour. In fruits, carotenoids accumulate in chromoplasts and render the yellow, orange and red colours of many crop and ornamental plants. The pathway of carotenoid biosynthesis in plants was biochemically well established by the mid-1960s. The genetic basis of variation in fruit colour due to alterations in carotenoid content has been established in tomato and, to a lesser extent, in pepper. Furthermore, in the past few years, genes encoding nearly all of the enzymes have been cloned and characterised, providing the tools for the eventual modification of carotenoid content in fruits.

In contrast to flavonoids and carotenoids, which are widespread in the plant kingdom, betalains are found exclusively in one group of angiosperms, the *Caryophyllales* (including beetroot and *Amaranthus*). Betalains are derivatives

of betalamic acid that are present in the vacuoles of the cells of these plants giving colour, from yellow to red-purple, to different plant parts. The information on the metabolic pathways leading to the synthesis of these compounds and, consequently, knowledge about the involved genes is very scarce. This rules out at present any possibility of modifying by genetic engineering the content of these compounds in different plant tissues.

## 11.4 Enhancing fruit pigments: flavonoids

Among flavonoids, the anthocyanins are the most relevant compounds in term of fruit and flower colour. However, the final colour in a plant tissue is modified by the presence of metals, by pH, and by interactions of anthocyanins with colourless flavonoids (a phenomenon called co-pigmentation). Anthocyanins are subdivided into three groups of compounds (Fig. 11.2), the glycosylated derivatives of pelargonidin, cyanidin, and delphinidin, each one having a specific colour, brick-red, red, and blue respectively, that are distinguished by the hydroxylation pattern of the B ring. The modification of their balance within a specific plant tissue has been the objective of plant biotechnologists. However, most of the research reported so far has been applied to the modification of colour in the petals of flowers. This has been determined not only by the commercial value of flowers with novel colours, but also for technical reasons. Genetic transformation in flowers is easier than in other plant organs due to the availability of organ-specific promoters.

Natural mutations in genes that encode for enzymes of the flavonoid biosynthetic pathway result in the accumulation of pathway intermediates, giving new flower colours. However, no single species displays all the possible flower colours. Thus, species such as roses do not synthesise the purple delphinidin derivatives and therefore there are no blue roses. Similarly, orange pelargonidin glycosides are not found in *Petunia*, because the enzyme dihydroflavonol 4-reductase (DFR) of this species does not accept the colourless dihydrokaempferol as a substrate to produce pelargonidine (Fig. 11.2). However, transgenic *Petunia* plants were obtained which were transformed with a gene from maize encoding for another DFR enzyme, which could catalyse this transformation. The result has been the presence in the new transgenic plant of flowers with a brilliant brick red-orange colour unknown among the flowers of this species (Glick and Pasternak 1998). Modification by genetic engineering of the expression of a single gene may also function in the opposite direction, i.e., by suppression of its endogenous expression, which causes the blockade of the metabolic pathway where the enzyme encoded is involved. As a result, there is no production of the end product of the metabolic pathway which, if coloured, determines the absence or reduction of the plant tissue pigmentation. This has been the case reported for *Chrysanthemum* flowers with reduced pigmentation after silencing the endogenous *CHS* gene, encoding for the first enzyme of the pathway (Fig. 11.1). Therefore, silencing of the endogenous gene by genetic



engineering caused a diminished level of the CHS enzyme in the transgenic plant. This channelled the 4-coumaroyl-CoA intermediate to alternative routes other than the flavonoid biosynthetic pathway (Fig. 11.1).

It is common that modification of a single gene is not sufficient, by itself, to engineer a plant with a modified colour. For instance, it has been recently reported that introduction in carnation of a *flavonoid 3'5'hydroxylase* gene (encoding F3'5'H enzyme, Fig. 11.1) from *Petunia* needed the introduction of another gene to change the colour of the carnation flower from red to deep purple. The second gene was one encoding a cytochrome b5 protein. Apparently, the function of this second protein as an electron donor is critical for the NADPH:cytochrome P450 reductase that associates with the 3'5'hydroxylase (F3'5'H). The point is that the regulation of a complex metabolic pathway, such as the synthesis of anthocyanins (Figs 11.1 and 11.2), is not simple. The balance among metabolic intermediates and enzymes is systemically regulated. This means that a change in one metabolic step is followed by changes in the complete pathway in order to be adjusted to the new situation. Since all the elements of the complete system are not often known, the result is that a genetic engineering approach may be uncertain, and the results obtained unexpected. This was the case reported on *Petunia* plants transformed with a chalcone reductase (CHR) gene from *Medicago sativa* (Fig. 11.1). This CHR activity yielded a different chalcone from those produced by the endogenous *Petunia* CHR and it was not a natural substrate for the enzyme chalcone isomerase (CHI) of this species. In *Petunia*, the endogenous chalcones are ephemeral intermediates in the flavonoid metabolism and therefore do not accumulate. However, the new intermediate and related yellow derivatives were accumulated in the transgenic plants. The final result was the production of yellow flowers in a naturally uncoloured *Petunia* variety (Davies *et al.* 1998). Some transformation in deep-purple-flowered varieties also caused some redirection of intermediates in the transgenic plants since the final result was a reduction in the degree of purple pigmentation.

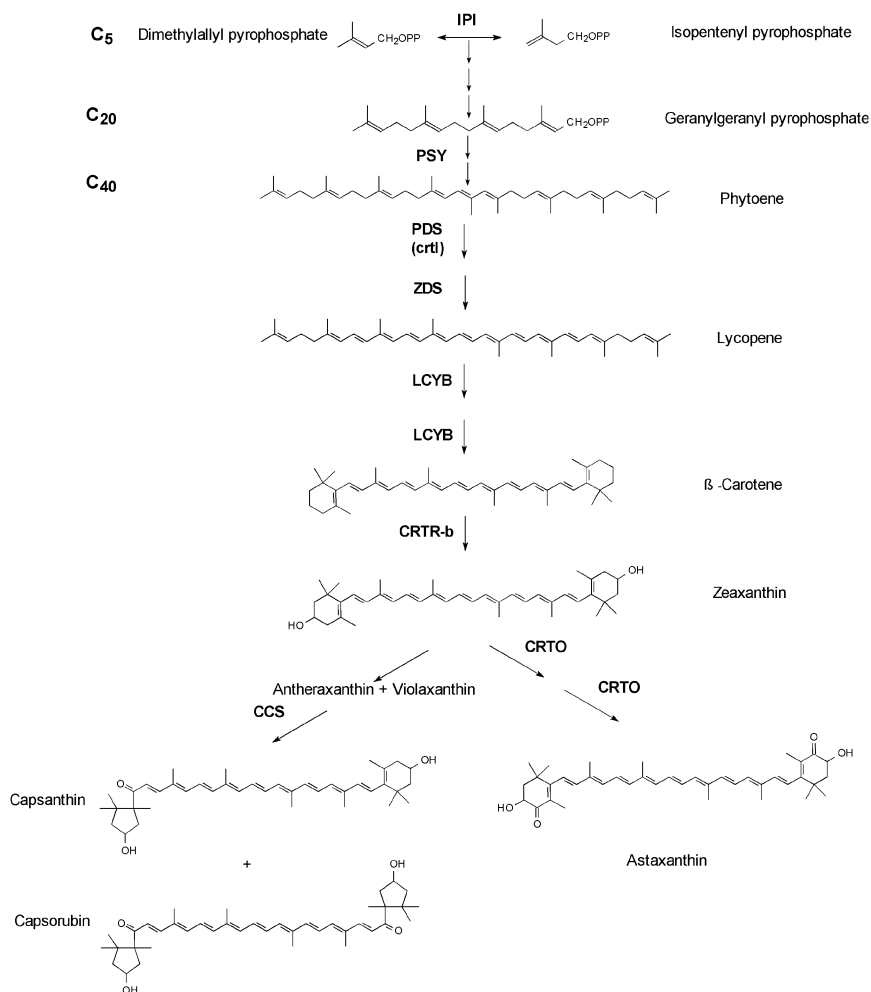
The difficulty of changing the concentration of the end product of a metabolic pathway by modification of a single (or several) steps of the complete pathway, could be addressed with a different strategy. Regulatory genes have the ability to switch on entire pathways or particular branches of a pathway. Thus, targets for genetic engineering have been the regulatory genes, i.e., those genes whose protein products, known as transcription factors, control the expression of other genes. As an example, the R and C1 genes activate the entire anthocyanin biosynthetic pathway in maize. Ectopic expression of these genes in maize was sufficient to induce anthocyanin synthesis in otherwise unpigmented tissues. When the same genes were introduced into dicots plants, such as *Arabidopsis* and tobacco, anthocyanin pigmentation was increased in petals and ectopically, only in roots and stamens (Lloyd *et al.* 1992). Further studies in dicots have shown that regulatory control is more complex in these species. Thus, production of anthocyanins by forced expression of R and C1 genes may be feasible in plant species with maize-type control.

In fruits, there has recently been reported tomato plants whose colour has been slightly modified as a side-effect of the modification of the level of flavonols. Flavonols are very potent antioxidants and it has been shown that a high intake is correlated to a decreased risk of cardiovascular disease. The biotechnological goal was to increase the level of flavonols in the edible part of the plant, the fruit, after knowing the health benefits of these flavonols. This involved transformation of tomato with a *Petunia* chalcone isomerase gene (*CHI*) which caused a 78-fold increase in fruit peel flavonols, mainly quercetin glycosides (Muir *et al.* 2001). As result, there was no accumulation of the yellow intermediate chalcone (Fig. 11.1), and this could explain a reduction of brightness in the tone of the red colour in the transgenic ripe fruits.

## 11.5 Enhancing fruit pigments: carotenoids

The carotenoid biosynthetic pathway takes place in the plastids of plants. The starting building block for all isoprenoids is the 5-carbon ( $C_5$ ) compound isopentenyl pyrophosphate (IPP, Fig. 11.3). This can be isomerised by the enzyme IPP isomerase (IPI) to produce dimethylallyl pyrophosphate (DMAPP), the activated substrate for formation of longer chain compounds. The precursor of carotenoids in plants is the  $C_{20}$  geranylgeranyl diphosphate (GGDP), which is also the precursor of other valuable metabolites such as vitamins E and  $K_1$  (Cunningham and Gantt 1998). The enzyme phytoene synthase (PSY) catalyses the condensation of two molecules of GGDP to produce the  $C_{40}$  colourless phytoene, the starting compound specific to the metabolic route for carotenoids (Fig. 11.3). This carotenoid undergoes four desaturation steps, catalysed by two related enzymes, phytoene desaturase (PDS) and  $\zeta$ -carotene desaturase (ZDS), to form the red-coloured lycopene, which is further transformed in the yellow-coloured  $\beta$ -carotene by the action of the lycopene- $\beta$ -cyclase (LCYB) (Fig. 11.3).  $\beta$ -Carotene is the major dietary precursor of vitamin A and therefore represents a fundamental component in our diet. The later steps of carotenoid biosynthesis in plants involve the formation of xanthophylls, which are oxygenated derivatives. Among these, capsanthin results from the activity of a bifunctional enzyme, the capsanthin-capsorubin synthase (CCS), that catalyses the conversion of the ubiquitous antheraxanthin and violaxanthin, into capsanthin and capsorubin (Fig. 11.3).

The fruits of tomato and pepper constitute a good source of carotenoids, being lycopene in tomato and capsanthin in pepper, the main components accumulated in the plastids of the ripe fruits. In tomato, total carotenoid content increases 10–15-fold during fruit ripening and the genes encoding PSY, PDS and LCYB have been identified as appropriate targets for genetic engineering. In a first attempt to modify the lycopene content of tomato fruits an additional copy of the tomato PSY gene was introduced in transgenic plants. The unexpected result was a reduction in the level of the endogenous phytoene synthase gene as a result of the transformation (Fray *et al.* 1995). It is not uncommon that the



**Fig. 11.3** Simplified diagram of isoprenoid metabolic pathway relative to the conversions among carotenoids that have been genetically manipulated. Enzymes catalysing some key reactions are indicated by the following abbreviations: IPI, isopentenyl pyrophosphate isomerase; PSY, phytoene synthase; PDS, phytoene desaturase (crtI, gene from *Erwinia uredovora*); ZDS,  $\delta$ -carotene desaturase; LCYB, lycopene  $\beta$ -cyclase; CRTR-b,  $\beta$ -carotene 3,3'-hydroxylase; CCS, capsanthin-capsorubin synthase; CRTO,  $\beta$ -carotene ketolase.

transformation of a plant with an additional copy of an endogenous gene causes the silencing of its own gene. This phenomenon is known as co-suppression. In the case reported, the final result was a decrease in the carotenoid content in the tomato fruit and a redirection of the intermediate metabolites of the pathway with deleterious effects on the transgenic plant. Another work known is the transformation of tomato with a bacterial phytoene desaturase gene, *crtI* from *Erwinia uredovora* (Römer *et al.* 2000). The result was somehow unexpected

since the fruits of the transgenic plants had threefold increase in  $\beta$ -carotene rather than lycopene, which is the product of the phytoene desaturase activity. Therefore, the increased production of lycopene in the transgenic plant may have upregulated the endogenous lycopene cyclase to produce  $\beta$ -carotene. Interestingly, the nutritional value of the fruits was improved since the compound  $\beta$ -carotene is the precursor of vitamin A. As a consequence of the change in the lycopene/ $\beta$ -carotene ratio, fruit colour was also altered in the transgenic tomatoes. Therefore transgenic plants with higher levels of  $\beta$ -carotene were orange and those with a moderate increase in  $\beta$ -carotene had an orange-red colouration compared to untransformed red tomatoes.

Other researchers have sought to alter endogenous lycopene- $\beta$ -cyclase activity (LCYB) in transgenic tomato plants (Rosati *et al.* 2000). The overexpression and the silencing of the endogenous gene were achieved by using different strategies. Overexpression was obtained when the tomato plants were transformed with the lycopene- $\beta$ -cyclase gene from *Arabidopsis*. Silencing was obtained when the introduced DNA included the terminal 3'-portion of the tomato  $\beta$ -cyclase gene in antisense orientation. Plants transformed over-expressing the  $\beta$ -cyclase gene showed a significant increase in the  $\beta$ -carotene content. Fruits displayed different colours ranging from red to orange, depending upon the lycopene/ $\beta$ -carotene ratio. The antisense strategy caused up to 50% inhibition of the endogenous  $\beta$ -cyclase gene expression in fruits, but only a slight increase in the lycopene content.

It is worth mentioning here the case of the transgenic golden rice (Ye *et al.* 2000). This species was engineered to produce  $\beta$ -carotene in the endosperm of the seeds with the objective of fortifying the grain of this important crop in an essential nutrient as  $\beta$ -carotene (provitamin A). Rice plants do not produce  $\beta$ -carotene and are only able to synthesise geranylgeranyl diphosphate. Therefore, the production of  $\beta$ -carotene in rice required the transformation of three genes: a daffodil phytoene synthase, a bacterial phytoene desaturase (crtI), and a lycopene  $\beta$ -cyclase from *Narcissus pseudonarcissus*. Therefore, the complete  $\beta$ -carotene pathway was introduced in the rice genome, with adequate promoters and signal peptides to direct its expression in the endosperm. In addition to a high content of  $\beta$ -carotene, a golden-yellow colour was apparent in the endosperm of the transgenic rice lines that distinguished them from the untransformed plant lines.

Although pepper is an important crop whose colour is mainly determined by carotenoids, the availability of transgenic plants with modified carotenoid composition has not been reported. The main reason is the recalcitrancy of pepper to be transformed and regenerated by procedures that use *Agrobacterium* and standard plant organogenesis protocols. However, there is solid genetic evidence to assign a main role in the colour determination of the pepper fruit to the gene encoding the capsanthin-capsorubin synthase activity (CCS) (Bouvier *et al.* 1994).

Efforts have also been made to introduce genes in plants to produce carotenoids other than those that are already present in the transformed species.

At present, this genetic modification has been reported only in the model plant tobacco (Mann *et al.* 2000). This plant has been engineered to produce the red carotenoid astaxanthin (Fig. 11.3). In nature, this compound is synthesised by marine bacteria and microalgae and then passed on to fish through the food chain. It provides the pink colour to salmon, trout, and shrimp. Astaxanthin is a ketocarotenoid synthesised from  $\beta$ -carotene in a two-step conversion catalysed by the enzyme  $\beta$ -carotene ketolase (CRTO) (Fig. 11.3). The corresponding gene (*CrtO*) has been isolated from the unicellular green alga *Haemotococcus pluvialis*. Expression of the  $\beta$ -carotene ketolase gene from *H. pluvialis* in the nectary tissue of transgenic tobacco plants, that normally synthesise  $\beta$ -carotene and xanthophylls, caused an accumulation of novel ketocarotenoids changing the colour of the nectary from yellow to red.

## 11.6 Future trends

Because of the commercial value of flowers and fruits, pigmentation has been a subject of basic and applied research for several centuries and new varieties have been produced by traditional breeding techniques (i.e., continuous crossing and selection). The impressive results are obvious to anyone who visits flower exhibitions, garden centres or marketplaces. Flowers have an enormous range of colours and patterns and tomatoes or strawberries are a darker red and larger than they used to be. Today, however, transgenic approaches have also been introduced in our research. This allows fine engineering of metabolic pathways, avoiding the inconvenience of crossing detrimental characters associated with the selected ones during traditional breeding.

New types of foods are increasingly being seen on the shelves of health stores and supermarkets. These new foods with enhanced nutritional or health properties, the so-called nutraceuticals, are becoming more popular among consumers. Coloured compounds, by their antioxidant character and/or their role as vitamin precursors, have been known to provide health benefits when present in the human diet. Their increased levels in fresh and processed foods, as well as their use as food additives, demand a cheap and easy supply of them. Very often, their production in biological systems such as microorganisms and plants appears as the best option for their industrial production.

The number of research articles in which the pigment content of flowers or fruits have been modified by genetic engineering is continuously increasing. As a consequence, our understanding of the regulation of the different pathways is also growing. The ability to switch entire pathways by ectopic expression of transcription factors suggests new approaches for genetic engineering. However, several but not all the factors that control pigment biosynthesis are known and in many cases their mechanism of action is poorly understood. Additionally, it is still not clear the effect that a regulatory gene from one species could have when transformed in other species. Nevertheless, given the efficiency of current research all these questions will be answered in the near future.

## 11.7 Sources of further information and advice

- National Biotechnology Information Facility: [www.nbif.org](http://www.nbif.org). A compendium of links to biotechnology sites, and a listing of research tools and software, with sections on biosafety, patent law, career planning and education.
- FDA/CFSAN Biotechnology: <http://vm.cfsan.fda.gov/~lrd/biotechm.html>. The biotechnology directory of the Center of Food Safety and Applied Nutrition of the US Food and Drug Administration that provides public access to topics on safety of food derived from genetically modified organisms.
- Facts on GMOs in the EU. [http://europa.eu.int/comm/dgs/health\\_consumer/library/press/press63\\_en.pdf](http://europa.eu.int/comm/dgs/health_consumer/library/press/press63_en.pdf). Official site of the European Commission with directives on genetically modified organisms: legislation, scientific committees, labellings, liability.

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# 12

## Food colorings

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### 12.1 Introduction

The appreciation of color and the use of colorants dates back to antiquity. The art of making colored candy is shown in paintings in Egyptian tombs as far back as 1500 BC. Pliny the Elder described the use of artificial colorants in wine in 1500 BC. Spices and condiments were colored at least 500 years ago. The use of colorants in cosmetics is better documented than colorants in foods. Archaeologists have pointed out that Egyptian women used green copper ores as eye shadow as early as 5000 BC. Henna was used to redden hair and feet, carmine to redden lips, faces were colored yellow with saffron and kohl, an arsenic compound, was used to darken eyebrows. More recently, in Britain, in the twelfth century, sugar was colored red with kermes and madder and purple with Tyrian purple.

Until the middle of the nineteenth century, the colorants used in cosmetics, drugs and foods were of natural origin from animals, plants and minerals. That changed with the discovery of the first synthetic dyestuff, mauve, by Sir William Henry Perkin in 1856. The German dyestuff industry rapidly developed a large number of 'coal tar' colorants and they rapidly found applications in the food and cosmetic industries. At the turn of the century, over 700 synthetic colorants were available for use in foods in the US. The potential for fraud and personal harm was obvious and horror stories abounded. For example, Marmion<sup>1</sup> described a situation where a druggist gave a caterer copper arsenite to make a green pudding for a public dinner and two people died. History is rife with anecdotes about adulteration of food, from the recipe for bogus claret wine<sup>2</sup> in 1805 to the attacks on synthetic colorants today. Two centuries ago, adulteration had become a very



sophisticated operation. Accum<sup>3</sup> commented, 'To such perfection of ingenuity has the system of counterfeiting and adulterating various commodities of life arrived in this country, that spurious articles are everywhere to be found in the market, made up so skilfully, as to elude the discrimination of the most experienced judges – the magnitude of an evil, which in many cases, prevails to an extent so alarming, that we may exclaim – There is death in the pot'. Many of the adulterants involved color and flavor. Elderberries and bilberries were added to wine. Copper acetate was used to color artificial tea leaves. Red lead was a colorant for cheese. Obviously government regulation was essential and this led to a long series of publications on the safety of colorants for food, drugs and cosmetics.

This chapter is devoted to a description of the chemistry, applications, and safety of the wide variety of natural and synthetic colorants available today. But another aspect has entered into consideration. Food safety of colorants has usually been considered to be a negative if we ignore the many benefits of making food more attractive in appearance. The recent meteoric rise of the nutraceutical industries has made it possible to claim health benefits for many categories of food including the colorants. Where appropriate, the health claims will be included in this chapter.

## **12.2 Food, drug and cosmetic colorants**

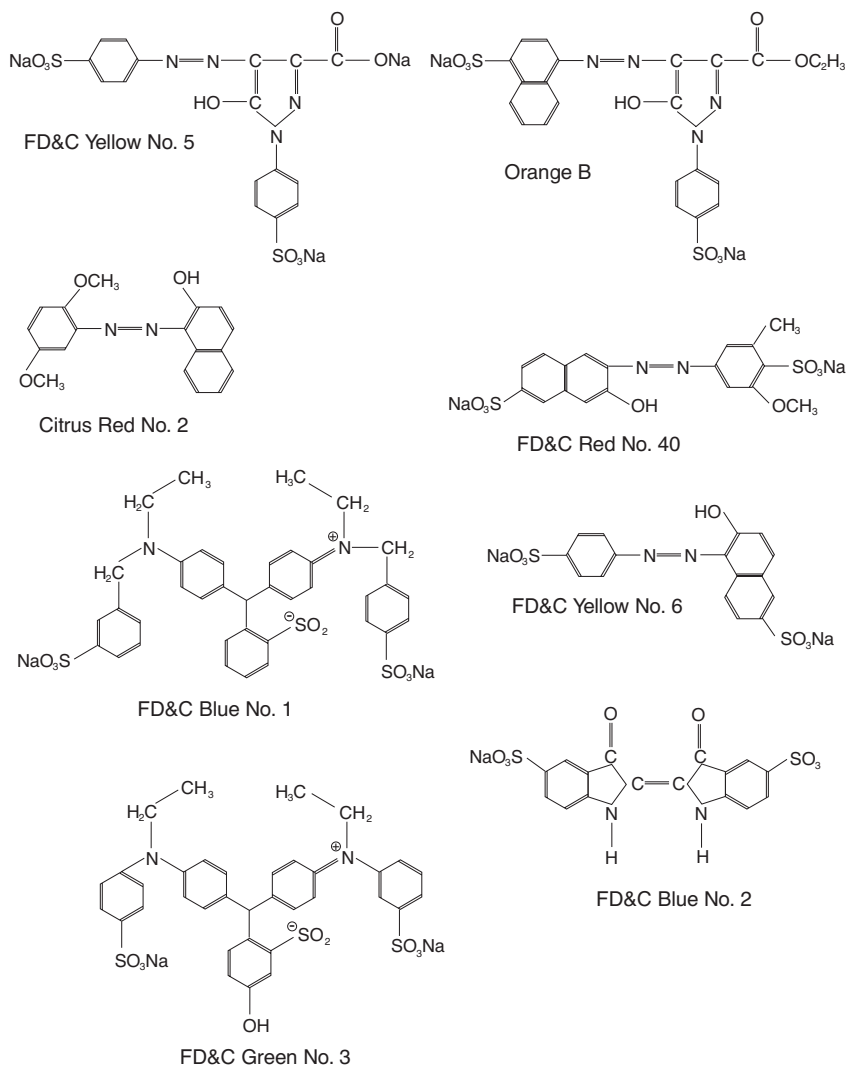
### **12.2.1 Introduction**

The chaotic situation existing in the synthetic colorant industry was evident in the 80 colorants available in 1907 to the paint, plastic, textile, and food industries. Obviously very few of them had been tested for safety. Dr Bernard Hesse, a German dye expert employed by the US Department of Agriculture, was asked to study the situation and he concluded that, of the 80 colorants available, only 16 were more or less harmless and he recommended only seven for use in food. This led to the US Food and Drug Act of 1906 which set up a certification procedure which ensured the identity of the colorant and the levels of impurities specifications for each food, drug and cosmetic (FD&C) color permitted for each colorant.<sup>4</sup>

The Federal Food, Drug, and Cosmetic Act of 1938 set up three categories:

1. FD&C colors for use in foods, drugs and cosmetics
2. D&C colors for use in drugs and cosmetics when in contact with mucous membranes or ingested
3. Ext. D&C colors for use in products applied externally.

The 1938 law required colorants on the permitted list to be 'harmless and suitable for food' but the FDA interpreted 'harmless' to mean harmless at any level and this proved to be unworkable. The Color Additive Amendment of 1960 eliminated the 'harmless per se' interpretation and resulted in a list of nine permitted colorants. The list was reduced to eight with the delisting of FD&C



**Fig. 12.1** Structures of eight permitted food, drug and cosmetic colorants.

Red No. 3 in 1998 (Fig. 12.1). Colorants in the three categories above were termed ‘certified’ colorants but the Color Additive Amendment also set up a category of ‘exempt’ colorants which were not subject to the rigorous requirements of the certified colorants. There are 26 colorants in this category (Table 12.1) and they comprise most of the preparations which would be called ‘natural’ in other countries. The US does not officially recognize the term ‘natural’ but it is often used in the popular press.

**Table 12.1** Regulatory and safety status of colorants exempt from certification in the US<sup>a</sup>

Color additive	US food use limit	EU status	JECFA ADI (mg/kg/bw)
Algal meal, dried	GMP <sup>b</sup> for chicken feed	NL <sup>c</sup>	NE <sup>d</sup>
Annatto extract	GMP <sup>e</sup>	E160b	0–0.065
Dehydrated beets	GMP	E162	NE
Ultramarine blue	Salt for animal feed up to 0.5//5 by weight	NL	None
Canthaxanthin	Not to exceed 30 mg/lb of solid/semisolid food or pint of liquid food or 4.41 mg/kg of chicken feed	E161g	None
Caramel	GMP	E150	0–200
Beta-apo-8-carotenal	Not to exceed 15 mg/lb of solid or semisolid food or 15 mg/pint of liquid food	E160a	0–5
Beta carotene	GMP	E150	0–5
Carrot oil	GMP	NL	NE
Cochineal extract or carmine	GMP	E120	0–5
Corn endosperm oil	GMP for chicken feed	NL	NE
Cottonseed flour,	GMP	NL	NE
Toasted partially defatted, cooked			
Ferrous gluconate	GMP for ripe olives only	NL	NE
Fruit juice	GMP	NL	NE
Grape color extract	GMP for non-beverage foods	E163	0–2.5
Grape skin extract (Enocianina)	GMP for beverages	E163	0–2.5
Iron oxide, synthetic	Pet food up to 0.25%	E172	0–2.5
Paprika	GMP	E160c	None
Paprika oleoresin	GMP	E160c	Self-limiting as a spice
Riboflavin	GMP	E101	0–0.5
Saffron	GMP	NL	Food ingredient
Tagetes meal and extract (Aztec Marigold)	GMP	NL	NE
Titanium dioxide	Not to exceed 1% by weight of food	E171	None
Turmeric	GMP	E100	Temporary ADI
Turmeric oleoresin	GMP	E100	Temporary ADI
Vegetable juice	GMP	NL	NE

<sup>a</sup>Adapted from Francis, F. J., 1999. Chap. 4, Regulation of Colorants in *Colorants*, Eagan Press. St. Paul, MN. pp. 223–32.

<sup>b</sup> Good Manufacturing Practice.

<sup>c</sup> Not listed.

<sup>d</sup> Not evaluated.

<sup>e</sup> Calculated as bixin.

### 12.2.2 Chemistry and usage

The certified and exempt colorants is the most important group of colorants in the US both from a poundage and diversity of applications point of view. The chemical structures of the certified group are shown in Fig. 12.1. This group also includes the lakes which are prepared by reacting the pure FD&C colorants with alumina and washing and drying the resultant precipitate.<sup>5</sup> FD&C colorants are available in many formulations: dry powders, solutions in a variety of solvents, blends, formulations with a variety of carriers, and lakes. The colorant preparations are used in a wide variety of foods available in the food markets. Lakes are used in food formulations where it is desirable to minimize the 'bleeding' of color from one ingredient to another. Stability and solubility of FD&C colorants under a variety of conditions was published by Marmion<sup>4</sup> and Francis.<sup>5</sup>

### 12.2.3 Safety

The history of the safety of the synthetic colorants has been replete with controversies and contradictions. The decisions by Bernard Hesse at the turn of the century were based primarily on the concept that the colorants should be harmless when consumed in everyday food. The authors of the 1938 Act were primarily concerned with developing a list of colorants which were allowed and would be harmless under the conditions of toxicological testing accepted in the 1930s. These included consideration of the anticipated levels of consumption with the levels of usage likely to be found across the range of expected products (the Acceptable Daily Intake, ADI). It became evident that this concept could be abused when, in 1950, some children became ill after eating popcorn with excessive levels of colorants. The FDA then launched a new round of toxicological investigations. The original list of seven colorants, which had grown to sixteen, was reduced to seven, and it included only two of the original seven. The toxicological protocols were based on weight changes, tumor production, biochemical and physical changes, etc. and teratological, immunological, multi-generation changes, allergenicity, carcinogenicity, and other changes were added. With the possible exception of saccharin, the FD&C colorants became the most tested group of additives in foods. The case of FD&C Red No. 2 (Amaranth) is an example. Amaranth was approved for use in foods in 1907 and survived the batteries of tests until a Russian study in 1972 concluded that it was carcinogenic. It was difficult to repeat the study because the Russian material was of textile grade and contained about 9% impurities, but a flurry of activity resulted. Two studies indicated a problem with carcinogenicity and 16 had negative effects. Regardless, FD&C Red No. 2 was delisted in 1976 in spite of the controversy over the interpretations. For example, the Canadian authorities allowed FD&C Red No. 2 to be used in foods and banned FD&C Red No. 40 (Allura Red) which largely replaced Red No. 2. The American authorities banned FD&C Red No. 2 and allowed FD&C Red No. 40. Similar discrepancies are found in the approved colorants for many

countries. A detailed discussion of the safety of each FD&C colorant<sup>6</sup> and the exempt colorants<sup>7</sup> was published but space does not allow a discussion of the safety considerations here. The general protocols for toxicological testing may be found in the FDA publication *Toxicological Principles for the Safety Assessment of Direct Food and Color Additives Used in Food*. Information on specific additives may be found in the *US Code of Federal Regulations – CFR 21*. Listings of the available colorants are also available.<sup>8,9</sup>

#### **12.2.4 Future prospects**

Synthetic colorants were severely criticized in the US in the 1960s by consumer groups critical of the ‘junk food’ concept. Activists had difficulty criticizing the foods themselves but the colorants which were essential to the formulation of convenience foods were vulnerable. The belief that natural additives were superior to synthetic compounds from a health point of view is a little naive since 5,000 years is too short a time for humans to develop genetic resistance. Regardless, the popularity of natural formulations increased worldwide and shows no signs of decreasing. The last synthetic food colorant to be approved by the FDA was FD&C Red No. 40 (Allura Red) in 1971 and there are not likely to be any more in the near future. A case can be made that there is no need for any more since, from a tristimulus approach with three primary colors such as red, blue and green, any color can be matched. This is not quite true since the current primary colors available do not cover the desired spectrum. Industry would like a wider choice and several new colorants are being considered.<sup>5</sup> Conventional wisdom would suggest that approvals for new colorants are likely to be in the natural group.

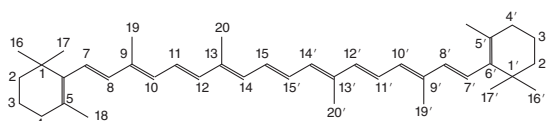
### **12.3 Carotenoid extracts**

#### **12.3.1 Introduction**

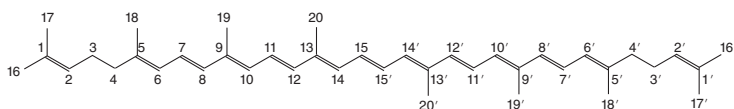
Carotenoids are probably the best known of the colorants and certainly the largest group of pigments produced in nature with an annual production estimated at 100,000,000 tons. Most of this is fucoxanthin produced by algae in the ocean and the three main pigments, lutein, violaxanthin and neoxanthin in green leaves.<sup>10</sup> Over 600 carotenoid compounds have been reported.

#### **12.3.2 Chemistry and usage**

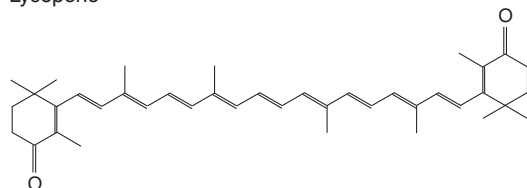
The chemical structure of some typical carotenoids is shown in Fig. 12.2. Beta-carotene occurs in nature usually associated with a number of chemically closely related pigments and extracts have been used as food colorants for many years. For example, palm oil has a high concentration of carotenoid pigments, primarily beta-carotene and about 20 others. Crude palm oil has been used extensively as a cooking oil because of its desirable flavor and as a general



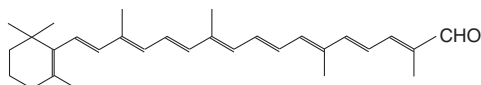
Beta-carotene



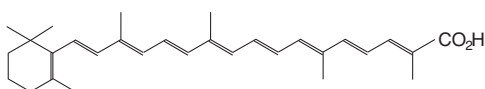
Lycopene



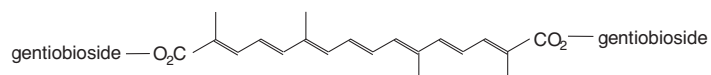
Canthaxanthin



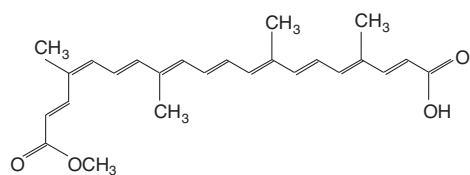
Beta-apo-8'-carotenol



Beta-apo-8'-carotenoic acid



Crocin



Bixin

**Fig. 12.2** Structures of some typical carotenoid compounds.

edible oil after purification. Both the crude oil and the semi-purified oil are effective colorants. Xanthophyll pastes, well known in Europe, consist of extracts of alfalfa (lucerne), nettles, broccoli and other plants. Unless saponified, they are green because of the chlorophyll content. Many xanthophyll pastes contain as much as 30% carotenes with the major pigments being those of green leaves, lutein, beta-carotene, neoxanthin, and violaxanthin. Extracts of carrots contain about 80% beta-carotene and up to 20% alpha carotene with traces of other compounds including lycopene. Extracts of citrus peels have been suggested for coloring orange juice since the more highly colored juices command a higher price. Astaxanthin is a desirable colorant for trout and salmon in aquaculture and the usual source is byproducts of the shrimp and lobster industries, but demand has led to the cultivation of the red yeast *Phaffia rhodozyma* to produce astaxanthin.<sup>11</sup> Extracts of marigold, *Tagetes erecta*, are well known commodities used primarily as colorants in poultry feed. Extracts are available in three main forms, dried ground petals, crude oleoresins and purified oleoresins in a wide variety of formulations. Preparations from tomatoes have been used to provide flavorful and colorful food ingredients for many years. Recent increases in the demand for natural beta-carotene has led to the production of beta-carotene extracts from microalgae, and some species of *Dunaliella* can accumulate up to 10% d.w. of beta-carotene.<sup>11</sup>

All carotenoid extracts are effective yellow to orange colorants and can be used in a variety of foods depending on government regulations. These include vegetable oils, margarine, salad dressings, pastas, baked goods, dairy products, yoghurt, ice cream, confectionery, juices, and mustard.

### 12.3.3 Toxicology

There is little toxicological data available for extracts of carrots, alfalfa, corn oil, palm oil, tomatoes, etc. The JECFA had no objections to their use as food colorants provided that the levels of use did not exceed that normally present in vegetables. A number of toxicity experiments were conducted on *Dunaliella* algae in view of its increasing importance in the health food area. Twelve studies on *D. salina* indicated no problems. *Cis* beta-carotene was absorbed to a lesser extent than *trans* beta-carotene. Furahashi suggested a no-observed-effect level (NOEL) of 2.5 g/kg/day for extracts from *D. Hardawil*.<sup>12</sup> The Joint Expert Committee on Food Additives of the World Health Organization/United Nations (JECFA) did not establish a NOEL or an ADI because of the variation in the composition of the products.

### 12.3.4 Health aspects

Carotenoids are of physiological interest because some of them are precursors of vitamin A. They have been in the news recently because many exhibit radical or single oxygen trapping ability and as such have potential antioxidant activity *in vivo*. They may reduce the risk of cardiovascular disease, lung cancer, cervical

dysplasia, age-related macular degeneration, and cortical cataract.<sup>13</sup> The beneficial effects of beta-carotene are thought to occur through one of several modes: singlet oxygen quenching (photoprotection), antioxidant protection, and enhancement of the immune response. Evidence suggests that a diet rich in carotenoids reduces the risk of coronary heart disease but supplementing the diet with synthetic beta-carotene did not produce the same benefit. Possibly other carotenoids are important in the diet and this has led to increased interest in carotenoids such as lutein. Interest in lycopene has increased dramatically in recent years due to epidemiological studies implicating lycopene in the prevention of cardiovascular disease, and cancers of the prostate and gastrointestinal tract.

### **12.3.5 Future prospects**

The dramatic increase in the health aspects of the carotenoids has spurred a great deal of interest in these compounds as colorants. The prospect of having both a health and a colorant aspect is very appealing to merchandisers so we can expect an increase in the number of carotenoid extracts available. But with over 600 carotenoids existing in nature, it will be difficult to determine which compounds exhibit health effects.

## **12.4 Lycopene**

### **12.4.1 Introduction**

Lycopene is the major pigment in tomatoes and is one of the major carotenoids in the human diet. It also accounts for 50% of the carotenoids in human serum. Tomato products are widespread in diets around the world and are highly prized for their flavor and color contributions.

### **12.4.2 Chemistry and usage**

The major source of lycopene is tomato products but it also occurs in water melons, guavas, pink grapefruit, and in small quantities in at least 40 plants.<sup>14</sup> The structure of lycopene is shown in Fig. 12.2. It is a long chain conjugated hydrocarbon and its structure suggests that it would be easily oxidized in the presence of oxygen and isomerized to *cis* compounds by heat. Both of these reactions occur in purified solutions of lycopene but in the presence of other compounds normally present in tomatoes, lycopene is more stable. Actually the absorption of lycopene in the human gut is increased by heat treatment probably because the breakdown of the plant cells makes the pigment more accessible. Preparations from tomatoes are widely used in pizza, pasta, soups, drinks and any product compatible with the flavor and color of tomatoes.



#### **12.4.3 Toxicology**

There is little safety data available for tomato products probably because they have been a major food for so long.

#### **12.4.4 Health aspects**

A recent review of 72 independent epidemiology studies revealed that intake of tomatoes and tomato products was inversely related to the risk of developing cancers at several sites including the prostate gland, stomach and lung. The data were also suggestive of a reduced risk for breast, cervical, colorectal, esophageal, oral cavity and pancreas.<sup>13</sup> Obviously, the role of lycopene is going to get more research attention in the future.

### **12.5 Lutein**

#### **12.5.1 Introduction**

Lutein is a major component of many plants. It is a component of most of the carotenoid extracts suggested as food colorants.

#### **12.5.2 Chemistry and usage**

Lutein has a structure similar to beta-carotene with a hydroxyl group on the ionone ring at each end of the molecule. It is somewhat less sensitive to oxidation and heat degradation than beta-carotene. It contributes a yellow color.

#### **12.5.3 Toxicology**

Little data is available but it would be expected to be non-toxic by comparison to similar carotenoids.

#### **12.5.4 Health effects**

Several studies have linked lutein to a lower risk for eye, skin and other health disorders, probably through its antioxidant activity. Lutein is apparently metabolized to zeaxanthin, an isomer, and several other compounds which protect the macula from ultraviolet radiation. The suggestion is that lutein may play a positive role in reducing macular degeneration. Other reports have linked lutein to a reduction of risk of cancer.<sup>13</sup> Regardless, lutein is currently being promoted as an important dietary supplement.

## 12.6 Annatto and saffron

### 12.6.1 Introduction

Annatto is one of the oldest colorants, dating back to antiquity for coloring food, cosmetics and textiles. Annatto is produced from the seeds of the tropical shrub *Bixa orellana*. Saffron is also a very old colorant dating back to the 23rd century BC. It is produced from the dried stigmas of the flowers of the crocus bulb, *Crocus sativa*. Saffron is known as the gourmet spice because it produces a desirable flavor and color. Its high price is assured because it takes about 150,000 flowers to produce one kilogram of saffron.

### 12.6.2 Chemistry and usage

The main pigments in annatto are bixin (Fig. 12.2) and norbixin. Bixin is the monomethyl ester of a dicarboxyl carotenoid. Norbixin is the saponified form, a dicarboxyl acid of the same carotenoid. The carboxylic acid portion of the molecule contributes to water solubility and the ester form contributes to oil solubility. Annatto is available in both water soluble and oil soluble liquids and powders. Annatto is somewhat unstable to light and oxygen but, technically, it is a good colorant. The principal use of annatto is as colorant for dairy products due to its water solubility but it is also used to impart a yellow to red color in a wide variety of products. The main pigments in saffron are crocin (Fig. 12.2) and crocetin. Crocin is the digentiobioside of the dicarboxylic carotenoid crocetin. The carboxylic and the sugar portion of the molecule contribute to water solubility. It is more stable to light and oxygen than annatto but, technically, it is a good colorant and is used in a variety of gourmet foods.<sup>15</sup>

### 12.6.3 Toxicology

A series of studies have shown annatto to be non-genotoxic<sup>11,15</sup> but others have suggested some mitotic aberrations<sup>16</sup> and some genotoxicity.<sup>17</sup> The acute oral toxicity is very low. The oral LD<sub>50</sub> for rats is greater than 50 g/kg for the oil soluble form and 35 g/kg for the water soluble form. Lifetime toxicity studies in rats at the level of 26 mg/kg/day showed no toxic effects. Rats showed no reproductive problems when fed at 500 mg/kg/day for three generations. It was concluded that annatto was not carcinogenic. The JECFA established an ADI of 0–0.065 mg/kg/day for annatto based on studies nearly 40 years ago. Current research is under way to increase the ADI. Little data is available for saffron but the chemical similarity to the pigments in annatto, and other carotenoids, would suggest that saffron would pose no problems in the food supply.

### 12.6.4 Health effects

Annatto seeds have long been used by the South American Indians as a traditional medicine for healing of wounds, skin eruptions, healing of burns, and

given internally for diarrhea, asthma and as an antipyretic.<sup>15</sup> Annatto is claimed to have strong antioxidative potency, as shown by inhibition of lipid peroxidation and lipoxidase activity.

### 12.6.5 Future prospects

Annatto is well established in the market and its use is increasing in poundage probably due to its superior technological properties. If some of the health claims prove to be true, annatto will enjoy increased interest. Saffron is well established in the gourmet markets but its use will be restrained because of its high price.

## 12.7 Paprika

### 12.7.1 Introduction

Paprika is a very old colorant and spice. It is a deep red, pungent powder prepared from the dried pods of the sweet pepper, *Capsicum annum*.

### 12.7.2 Chemistry and usage

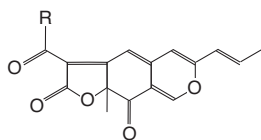
Paprika contains capsorubin and capsanthin (Fig. 12.3) which occur mainly as the lauric acid esters, and about 20 other carotenoid pigments. Paprika is produced in many countries which have developed their own specialties. Cayenne or cayenne pepper, produced from a different cultivar of *C. annum*, is usually more pungent. *C. frutescens* is the source of the very pungent Tabasco sauce. Paprika oleoresin is produced by solvent extraction of the ground powder. Obviously paprika supplies both flavor and color and its use is limited to those products compatible with the flavor. The recent rise in demand for tomato products in the form of pizza, salsa, etc., has increased the demand for paprika. Paprika is used in meat products, soups, sauces, salad dressings, processed cheese, snacks, confectionery and baked goods.<sup>10,18</sup>

### 12.7.3 Toxicology

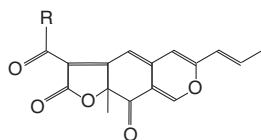
The acute oral toxicity of paprika is very low with an LD<sub>50</sub> for mice of 11 g/kg. Several studies have indicated that paprika is not genotoxic. The JECFA did not establish an ADI because they considered that the levels of paprika and its oleoresins in foods would be self-limiting.<sup>11</sup>

### 12.7.4 Future prospects

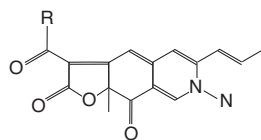
Paprika is well established worldwide and will probably increase in volume due to the popularity of tomato products and possibly by analogy to the health effects being attributed to the carotenoids.



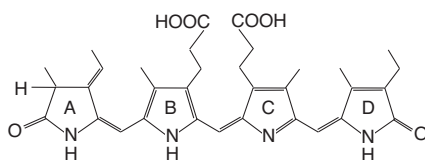
Monascin  $R = C_5H_{11}$   
Ankaflavin  $R = C_7H_{15}$



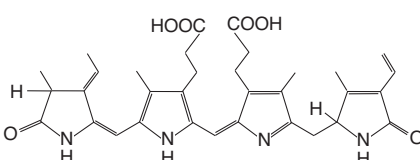
Rubropunctatin  $R = C_5H_{11}$   
Monascorubin  $R = C_7H_{15}$



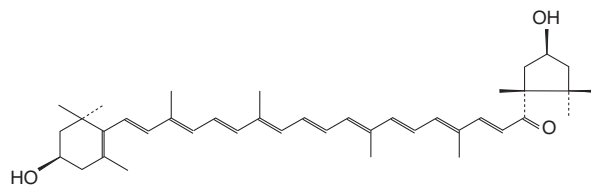
Rubropunctamine  $R = C_5H_{11}$   
Monascorubramine  $R = C_7H_{15}$



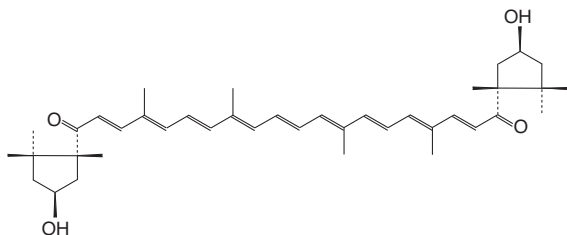
Phycocyanobilin



Phycoerythrobilin



Capsanthin



Capsorubin

**Fig. 12.3** Top. Pigments in *Monascus*. Middle. Phycobilins in algae. Bottom. Pigments in paprika.

## 12.8 Synthetic carotenoids

### 12.8.1 Introduction

The success of the carotenoid extracts led to the commercialization of synthetic carotenoids, some with the same chemical structure as those in the plant extracts and others with modifications to improve their technological properties. The yellow beta-carotene was synthesized in 1950, followed by the orange beta-8-carotenol in 1962 and the red canthaxanthin in 1964. A number of others soon followed, methyl and ethyl esters of carotenoic acid, citraxanthin, zeaxanthin, astaxanthin, and recently lutein.

### 12.8.2 Chemistry and usage

The structures of four of the synthetic carotenoids (beta-carotene, canthaxanthin, beta-apo-8'-carotenol, beta-apo-8'-carotenoic acid) are shown in Fig. 12.2. By virtue of their conjugated double bond structure, they are susceptible to oxidation but formulations with antioxidants were developed to minimize oxidation. Carotenoids are classified as oil soluble but most foods require water soluble colorants; thus three approaches were used to provide water dispersible preparations. These included formulation of colloidal suspensions, emulsification of oily solutions, and dispersion in suitable colloids. The Hoffman-LaRoche firm pioneered the development of synthetic carotenoid colorants and they obviously chose candidates with better technological properties. For example, the red canthaxanthin is similar in color to lycopene but much more stable. Carotenoid colorants are appropriate for a wide variety of foods.<sup>10</sup> Regulations differ in other countries but the only synthetic carotenoids allowed in foods in the US are beta-carotene, canthaxanthin, and beta-8-carotenol.

### 12.8.3 Toxicology

When the first synthetic carotenoid colorant, beta-carotene, was suggested as a food colorant, it was subjected to an extensive series of tests<sup>11</sup> despite the belief that carotenoid extracts containing beta carotene were non-toxic. It was thought that synthetic preparations might contain a different profile of minor contaminants than those in plant extracts. Beta-carotene has a very low acute oral toxicity. The LD<sub>50</sub> for dogs is greater than 1000 mg/kg and a single intramuscular injection of 1000 mg/kg in rats had no significant effect. Lifetime dietary administration showed no carcinogenicity with a NOEL of 100 mg/kg/day for rats and 1000 mg/kg/day for mice. No teratogenic or reproductive toxicity was shown when four generations of rats were fed up to 100 mg/kg/day. No cytogenic or teratogenic effects were seen in the offspring of rabbits given 400 mg/kg/day by stomach tube. Similar experiments with rats given up to 1000 mg/kg/day by intubation showed no embryo toxicity, teratogenicity or reproductive effects. Apparently rodents can tolerate large amounts of beta-carotene but extrapolation to humans is difficult because rats and humans

metabolize beta-carotene in different ways. With humans, the absorbed beta-carotene is largely converted to vitamin A, esterified, and transported in the lymph. With rats, the beta-carotene is largely converted to non-saponifiable compounds. Regardless, there is ample evidence that beta-carotene in reasonable quantities is harmless to humans. There is, however, evidence that humans with a high intake of beta-carotene may develop hypercarotenemia leading to an orange skin coloration. The JECFA established an ADI of 0–5 mg/kg/day for the sum of all carotenoids used as colorants.

Canthaxanthin was also subjected to an extensive series of toxicological tests<sup>11</sup> which indicated that it was essentially non-toxic. The acute oral toxicity was very low with an oral LD<sub>50</sub> for mice greater than 10 g/kg. No effect was seen in dogs fed 500 mg/kg/day for 15 weeks. Canthaxanthin was not carcinogenic at feeding levels of 1000 mg/kg/day for 104 weeks for rats and 98 weeks for mice and it may have been anti-carcinogenic. No reproductive or teratogenic effects were seen when rats were fed up to 1000 mg/kg/day for three generations. Ingestion of large amounts of canthaxanthin caused deposition of canthaxanthin crystals, producing retinal impairment, in the eyes of humans, cats and rabbits, but not in rats, mice and dogs. In humans the visual impairment was reversible in a few months. Effects of high doses are available because canthaxanthin is used as a tanning aid. Other adverse effects were hepatitis and urticaria. The JECFA was unable to assign an ADI because of the problem with retinal crystal deposition in humans. Canthaxanthin, in the amounts required for appropriate coloration, is believed to be completely safe.

Beta-8-carotenal and the methyl and ethyl esters of carotenoic acid were also tested for toxic effects with results similar to beta-carotene.

#### **12.8.4 Health effects**

There is considerable current research under way to determine if the synthetic carotenoids have the same physiological effects, and consequent health benefits, as the naturally occurring compounds. If this proves to be true, we can expect increased interest in this group of colorants.

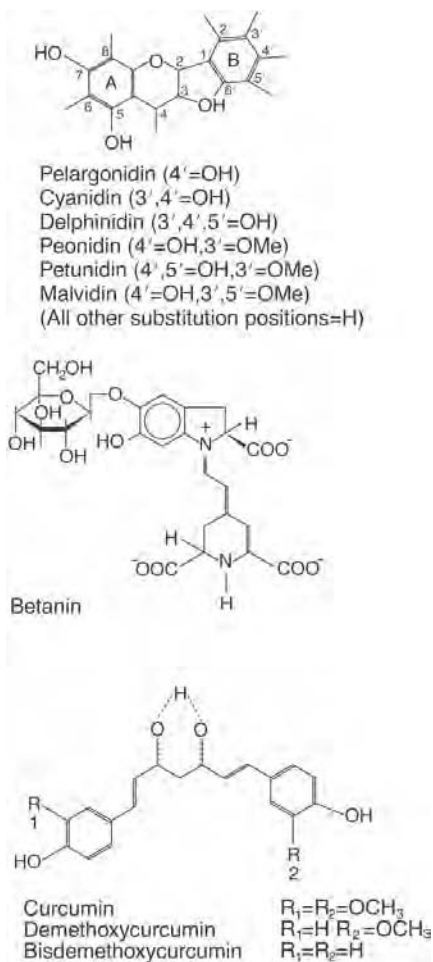
### **12.9 Anthocyanins**

#### **12.9.1 Introduction**

Anthocyanins are ubiquitous in the plant kingdom. They are responsible for many of the orange, red, blue, violet, and magenta colors. They have been the object of intensive research from a taxonomic point of view and this has resulted in about 275 known structures and about 5,000 of the chemically closely related flavonoid compounds. Their use as colorants dates back to antiquity since the Romans used highly colored berries to augment the color of wine.

### 12.9.2 Chemistry and usage

Anthocyanins consist of an aglycone combined with one or more sugars. Twenty-two aglycones are known but only six are important as colorants (Fig. 12.4). In view of the ubiquity and high tinctorial power it is not surprising that many sources have been suggested as colorants. Francis listed pigment profiles and methods of extraction for over 40 plants<sup>19,20</sup> and also 49 patents on anthocyanin sources as potential colorants.<sup>21</sup> However, despite the large number of sources, only one dominated the supply for many years. Colorants from grape skins as a by-product of the wine industry is the major source. Grapes are the largest fruit crop for processing and since 80% of the estimated 60,000,000



**Fig. 12.4** Top. Structures of the major anthocyanidins in foods. Middle. Structure of betanin. Bottom. Pigments in turmeric.

metric tons is used annually for wine production, this situation is not likely to change. Recently anthocyanins from red cabbage have enjoyed some success.

The sugars attached to the anthocyanin molecule are in order of relative abundance glucose, rhamnose, galactose, xylose, arabinose, and glucuronic acid. The molecule may also contain one or more of the acyl acids p-coumaric, caffeic, and ferulic or the aliphatic acids malonic and acetic esterified to the sugar molecules. Extracts of anthocyanins invariably contain flavonoids, phenolic acids, catechins and polyphenols. The net result is that it is impossible to express the chemical composition accurately. Specifications usually present tinctorial power, acidity, per cent solids, per cent ash and other physical properties.

The major market for the colorants from grapes (generic term enocyanin) is in fruit drinks. Anthocyanins are pH sensitive and show the greatest tinctorial power around pH 3–3.5 and most fruit drinks are in this range. Anthocyanin colorants have been used in a wide variety of food products such as beverages, jams, jellies, ice cream, yoghurt, gelatin desserts, canned fruits, toppings, confections, and many others.

### 12.9.3 Toxicology

Anthocyanins are not genotoxic as shown by a number of studies.<sup>19</sup> The oral toxicity of mixed anthocyanins was greater than 20 mg/kg/day for rats. Dogs fed a diet containing 15% anthocyanins from Concord grapes for 90 days showed no significant toxic effects. Another multigeneration study on rats fed 15% powder from Concord grapes showed no effects on reproduction. A study on Roselle, a popular drink made from the calyces of *Hibiscus sabdariffa*, showed no toxic effects.<sup>22</sup> The lack of toxic effects is not surprising in view of the long history of wine consumption.

### 12.9.4 Health aspects

Anthocyanins and the related flavonoids have been very much in the news lately for a wide variety of health claims. These include anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, antiallergenic, anti-thrombotic, and antioxidant effects.<sup>13</sup> Antioxidation is believed to be one of the most important mechanisms for preventing or delaying the onset of major diseases of aging including cancer, heart disease, cataracts and cognitive disfunction. The antioxidants are believed to block oxidative processes and free radicals that contribute to the causation of these chronic diseases. Anthocyanins are non-competitive inhibitors of lipid peroxidation comparable in potency to the classical antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and alpha tocopherol. Anthocyanins were reported to have anti-inflammatory properties comparable to commercial pharmaceuticals. The property of anthocyanins to decrease the fragility and permeability of blood capillaries is common to many flavonoids and was the basis for the original



definition of Vitamin P by Szent Gyorgi in 1936. This claim has been commercialized by the marketing of extracts of bilberry (*Vaccinium myrtillus*).

Interest in the health effects of anthocyanins was piqued by the 'French paradox' in which the mortality from cardiovascular disease was lower than that predicted from the intake of dietary saturated fatty acids. The beneficial effects were greater in association with alcohol taken in the form of wine suggesting that there may be a protective effect of other components of wine. Needless to say the wine industry was pleased with this research.

### 12.9.5 Future prospects

The potential health effects of anthocyanins and flavonols has stimulated much research in this area but, in view of the chemical complexity of the plant extracts, we are a long way from determining the chemical compounds responsible for the wide variety of claims. Regardless, a colorant with associated health benefits is a very desirable situation from an industry point of view. This is a very active research area.

## 12.10 Betalains

### 12.10.1 Introduction

The betalains are confined to ten families of the order *Caryophyllales*.<sup>20</sup> The only foods containing betalains are red beet (*Beta vulgaris*), chard (*B. vulgaris*), cactus fruit (*Opuntia ficus-indica*) and pokeberries (*Phytolacca americana*). They also occur in the poisonous mushroom *Amanita muscaria* but this is not a normal food source. The importance of the betalains as colorants is confined to preparations from red beet.

### 12.10.2 Chemistry and usage

The betalains have two main groups; the red betacyanins and the yellow betaxanthins. The main pigment of red beets is betanin and its structure is shown in Fig. 12.4. Beets usually contain both betacyanins and betaxanthins and the ratio depends on the cultivar. Some cultivars contain only the yellow betaxanthins and this makes it possible to formulate a range of colorants from yellow to red. Betanin is relatively stable to changes in pH as contrasted with the anthocyanins and this makes it preferable for foods in the pH range 5–6. Both the red and yellow betalains are susceptible to degradation by heat, light and the presence of metal ions. Within these limitations, betalains are ideally used to color products that have a short shelf life, are packaged to reduce exposure to light, oxygen and high humidity, do not receive extended or high heat treatment, and are marketed in the dry state. Despite these limitations, betalains have been suggested for coloring ice cream, yoghurt, cake mixes, gelatin desserts, meat substitutes, gravies, frostings and many others.<sup>20,23</sup>

### 12.10.3 Toxicology

Betalain pigments have been tested on rodents by feeding 50 mg/kg pure betanin, 2000 ppm betanine in the diet and several other conditions.<sup>11</sup> No carcinogenic or other toxic effects were observed and the authors concluded that red beet extracts were safe as food colorants.

### 12.10.4 Future prospects

Betalain colorants are well established in the food chain and will probably continue in a limited capacity.

## 12.11 Chlorophylls

### 12.11.1 Introduction

The chlorophylls are a group of naturally occurring pigments produced in all photosynthetic plants including algae and some bacteria. Hendry<sup>24</sup> estimated annual production at about 1,100,000,000 tons with about 75% being produced in aquatic, primarily marine, environments. Obviously as a source of raw material for food colorants, chlorophylls present no problem with supply.

### 12.11.2 Chemistry and usage

Five chlorophylls and five bacteriochlorophylls are known but only two, chlorophylls a and b are important as food colorants. Chlorophyll a has a complex structure with a magnesium ion in the molecule which is easily removed in acid media to form pheophytin a. Removal of the phytol portion of the molecule produces chlorophyllide a whereas removal of both magnesium and phytol produces pheophorbide a. Chlorophyll b reacts in the same manner. Chlorophyll and chlorophyllide are both bright green in color but pheophytin is olive green and pheophorbide is brown. Attempts to produce a food colorant from chlorophyll are centered around trying to stabilize the molecule by retaining or replacing the magnesium ion. Treatment with copper and zinc salts substitutes copper or zinc for the magnesium and the derivatives are bright green in color. Commercial colorants are usually made from lucerne (alfalfa), *Medicago sativa*, or nettles, *Urtica dioica*, and a series of pasture grasses. The plants are dried, extracted with a solvent and dried resulting in a mixture of chlorophyll, pheophytin and other degraded compounds. The dry residue can be purified to obtain an oil-soluble preparation or treated with an acidified copper solution to prepare a more stable water-soluble copper chlorophyllin. It is not commercially feasible to prepare a colorant containing pure chlorophyll because of the instability of the molecule. The major portion of the chlorophyll colorants are in the water soluble forms and are used in dairy products, soups, oils, sugar confections, drinks and cosmetics.<sup>25</sup>

### 12.11.3 Toxicology

The JECFA classified chlorophyll under List A which means that the colorant has been fully cleared and its use is not limited toxicologically since when used with 'good manufacturing practice' does not represent a hazard to health. A subchronic oral toxicity study showed no adverse effects.<sup>26</sup>

### 12.11.4 Health aspects

Chlorophyllin is an effective antimutagenic agent and has been used as a dietary supplement to diminish the intensity of the uncomfortable side effects of cyclophosphamide treatments.<sup>13</sup> Cyclophosphamide is a potent antitumor agent and is used against many forms of cancer and other diseases. Chlorophyllin protects against radiation induced DNA single strand breaks possibly by its ability to scavenge  $\text{OH}^+$  and  $\text{ROO}^+$  groups.

### 12.11.5 Future prospects

Chlorophyllins are approved for use in Europe and Asia but only for dentifrices in the US. Since there are no other commercially available natural green colors, these colorants are attracting interest.

## 12.12 Turmeric

### 12.12.1 Introduction

Turmeric is a very old colorant produced from the rhizomes of several species of *Curcuma longa*, a perennial shrub grown in many tropical areas around the world.

### 12.12.2 Chemistry and usage

Turmeric is the ground dried rhizomes and contains three main pigments, curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Fig. 12.4) together with about four potent flavor compounds.<sup>27,28</sup> Turmeric oleoresins are prepared by extracting the dried ground rhizomes with a variety of chemical solvents and concentrating the resins perhaps with the addition of oils and other carriers. Turmeric and turmeric oleoresins are unstable to light and alkaline conditions and a number of substances are sometimes added to stabilize the molecule. Curcumin is insoluble in water but a water-soluble form can be made by complexing the compound with tin or zinc to form an intensely orange colorant but it is not allowed in most countries. The major applications of turmeric are to color cauliflower, pickles and mustard but it is also used in combination with annatto in ice cream, yoghurt, baked goods, oils, salad dressings and confectionery.

### 12.12.3 Toxicology

Turmeric has been subjected to a number of safety studies because its consumption in Europe, India and the Middle East is very high. Consumption in India was estimated at up to 3.8 g/day.<sup>11</sup> The acute oral toxicity is very low. The oral LD<sub>50</sub> for the oleoresin in rats and mice is greater than 10 g/kg and for curcumin in mice is greater than 2 g/kg. Rats fed turmeric for 52 weeks at 500 mg/kg/day showed no significant toxicity. Similar experiments with dogs and monkeys showed similar results. There was no evidence of carcinogenicity, reproductive toxicity or teratology. The JECFA did not allocate an ADI for turmeric because they considered it to be a food. A temporary ADI of 0.01 mg/kg for turmeric oleoresin and 0–0.1 mg/kg for curcumin was established in 1990.<sup>12</sup> In 1995, the temporary ADI for curcumin was increased to 1 mg/kg.

### 12.12.4 Health effects

It has been known since the early 1950s that turmeric had strong antioxidant effects with curcumin being the major compound responsible followed by demethoxycurcumin and bisdemethoxycurcumin. All three inhibit lipid peroxidation and have a positive anti-oxidant effect for hemolysis and lipid peroxidation in mouse erythrocytes.<sup>11</sup> Curry pills containing turmeric are being marketed as a prevention for colon cancer.<sup>29</sup>

### 12.12.5 Future prospects

Turmeric is well established in the food supply and if it is proven to have a health effect as well as a colorant and flavor component, its future would seem assured.

## 12.13 Cochineal and carmine

### 12.13.1 Introduction

Cochineal is a very old colorant. References go back as far as 5000 BC when Egyptian women used it to color their lips. It was introduced to Europe by Cortez who found it in Mexico. Production peaked around 1870 and then declined due to the introduction of synthetic colorants, but it is still a major commodity for Peru, Mexico, and the Canary Islands.<sup>25,30</sup>

### 12.13.2 Chemistry and usage

Cochineal extract is obtained from the bodies of the female cochineal insects, particularly *Dactylopius coccus* Costa, by treating the dried bodies with ethanol. After removal of the solvent, the dried residue contains about 2–4% carminic acid, the main colored component. The cochineal insects grow on cactus and,

since it takes about 50,000–70,000 insects to produce one pound of the colorant, production will always be labor intensive.

Solutions of carminic acid, at pH4 show a range of colors from yellow to orange depending on the concentration. When complexed with aluminum, a series of stable brilliant red hues ranging from 'strawberry' to 'blackcurrant' can be produced depending on the ratio of aluminum to carminic acid. Purified extracts of cochineal have been termed 'carmine' but the term usually refers to a lake of carminic acid with aluminum, calcium or magnesium. Carmine usually contains about 50% carminic acid. Carmine is considered to be technologically a very good food colorant. It is ideally suited to foods with a pH above 3.5 such as comminuted meat and poultry products, surimi, and red marinades. It is also used in a wide variety of other products such as jams, gelatin desserts, baked goods, confections, toppings, dairy products, non-carbonated drinks, and many others.

### 12.13.3 Toxicology

A number of studies have shown that cochineal extract and carmine are not carcinogenic.<sup>11</sup> Rats fed carmine up to 100 mg/kg/day showed only reduced growth at the higher levels. Other studies show no reproductive problems in a single generation study or reproductive/teratology problems in a multigeneration study. The JECFA assigned a combined ADI of 0–5 mg/kg/day for cochineal and carmine.

### 12.13.4 Future prospects

Carmine is well entrenched in the food industry and probably will remain there because of its superior technological properties. It is, however, a very labor intensive industry because of the hand harvesting of the insects with a consequent high price.

### 12.13.5 Related compounds

Carmine belongs to the anthraquinone class of compounds and several other chemically closely related compounds are also used as colorants.<sup>25</sup> Kermes is a well known colorant in Europe. It is obtained from the insects, *Kermes ilicis* or *Kermococcus vermilis*, which grow on oak trees. It contains kermisic acid, the aglycone of carminic acid, and its isomer ceroalbolinic acid. Its properties are very similar to carmine. Lac is a red colorant obtained from the insect *Laccifera lacca* which is found on several families of trees in India and Malaysia. The lac insects are better known for their production of shellac. They contain a complex mixture of anthraquinones. Alkanet is a red pigment from the roots of *Alkanna tinctoria* Taush and *Alchusa tinctoria* Lom. All three have been cleared for food use in Europe but not in the US.

## 12.14 Monascus

### 12.14.1 Introduction

Monascus colorants are well known in Asia and in Chinese medicine date as far back as 1590. The colorants are produced by several fungal species of the genus *Monascus* which grow readily on a number of carbohydrates, especially rice, but also on wheat, soybeans, corn and other grains. The Koji process involves inoculating the solid grain mass with the fungus, primarily *Monascus purpureus*, and drying the substrate to produce 'red rice' which is used as a colorant for many foods.<sup>31,32</sup>

### 12.14.2 Chemistry and usage

*Monascus* species produce six pigments (Fig. 12.3). Monascin and ankaflavin are yellow, rubropunctatin and monascorubin are red, and rubropunctamine and monascorubramine are purple. The pigments are very reactive and have been reacted with a variety of compounds such as polyamino acids, amino alcohols, chitin amines or hexamines, proteins, sugar amines, aminobenzoic acid and many others<sup>21,28</sup> to produce compounds with greater water solubility, thermostability and photostability than the parent compounds. The ability to grow on a solid substrate has led to a large body of data on optimization of pigment production in solid state fermentation and later in submerged fermentation.<sup>31</sup> The conditions of growth can be modified to optimize the ratio of the different pigments and also for several other compounds with health implications. *Monascus* pigments are soluble in ethanol and the derivatives are soluble in water. A range of colors from yellow to purple can be produced by manipulating the ratio of pigments and the pH. Their stability in neutral media is a real advantage. The colorants are suitable for processed meats, marine products, ice cream, jam, toppings, and tomato ketchup, and traditional oriental products such as koji, soy sauce, and kambuki. Their solubility in alcohol makes them appropriate for alcoholic beverages such as saki.

### 12.14.3 Toxicology

*Monascus* colorants have been consumed for hundreds of years and are believed to be safe for human consumption. Tests with a series of microorganisms have demonstrated no mutagenicity. No toxicity was observed in rodents or in fertile chicken eggs. The yellow pigments have an LD<sub>50</sub> for mice of 132 mg/20g. No ADI is available.<sup>11</sup> There has been some concern that some of the strains produced antibiotics which is obviously undesirable for a food colorant, but it is possible to choose strains of *Monascus* which do not produce antibiotics.

### 12.14.4 Health effects

A large number of reports describe the use of *Monascus* preparations in herbal medicines and food supplements. These include the suppression of tumor

production, regulation of immunoglobulin production, lowering of lipids in hyperlipidemia, and reduction of aminoacetaphen-induced liver toxicity by antioxidant action. One example would be Cholestin distributed by the Pharmanex company. It is obtained from red rice imported from China as a nutraceutical and contains a natural inhibitor of the rate-limiting synthesis of cholesterol. It is claimed to reduce total cholesterol and low-density lipoprotein and triglyceride levels while increasing high-density lipoprotein levels. A nutraceutical with a beneficial effect for heart disease is a very desirable product so Cholestin was soon followed by several other products which may have been produced to maximize the content of the active ingredient. One problem was that the active ingredient was identical to that in Mevacor, a prescription drug patented by Merck. The FDA ruled that Cholestin was a drug, not a food supplement, and banned it. The Pharmanex company sued the FDA and the courts made a temporary decision in favor of the company and reversed the ban.<sup>31</sup> This situation seems to permit an end run around the strict rules governing approval of prescription drugs by simply finding a natural source, manipulating the growing conditions to maximize the content of the drug, and calling the formulation a food supplement.

#### **12.14.5 Future prospects**

Monascin colorants are well entrenched in Asia, particularly China, Japan, and Taiwan and probably will continue to be an important product in view of their long history. They are not allowed in the US and there seems to be little interest in them. Certainly, the situation illustrated by the Cholestin debate will have to be settled, probably by new legislation, before a commercial firm petitions to have Monascin colorants permitted in the US.

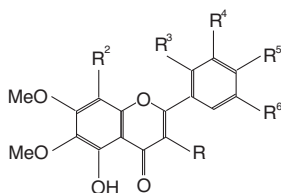
### **12.15 Iridoids**

#### **12.15.1 Introduction**

The colorants from saffron have enjoyed good technological success as colorants and spices but their high price has led to searches for other sources of the same pigments. The pigments, but not the flavor, can be obtained in much larger quantities from the fruits of the gardenia or Cape jasmine plant.<sup>33</sup>

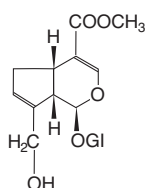
#### **12.15.2 Chemistry and usage**

The fruits of gardenia, *Gardenia jasminoides*, contain three groups of pigments, crocins, iridoids and flavonoids. Structures of six of the nine iridoid pigments are shown in Fig. 12.5. The formulas for five flavonoids from *G. fosbergii* are also shown. This is a different species but botanically closely-related species tend to have similar pigment profiles. The crocins are orange and the flavonoids are pale yellow. The iridoids are interesting because they can be reacted with amino acids or proteins to produce a range of colors from green to yellow, red,

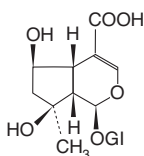


Flavonoid compounds

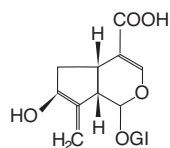
Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>
1	H	H	H	OMe	OMe	OMe
2	OMe	H	H	OH	OMe	OH
3	H	H	OMe	OMe	H	OH
4	OMe	H	H	OMe	OMe	OH
5	OMe	OMe	H	H	OH	H



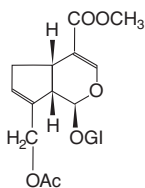
Geniposide



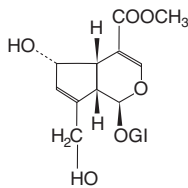
Shanzhiside



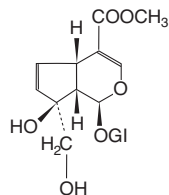
Gardoside



Acetylgeniposide



Methyldeacetylasperuloside



Gardenoside

**Fig. 12.5** Top. Some flavonoid pigments in gardenia. Bottom. Six of the nine iridoid pigments in gardenia.

and blue. A number of patents<sup>21</sup> have described the manipulation of the reaction conditions such as time, temperature, pH, oxygen content, degree of polymerization, reaction with selected microorganisms, etc. The compounds can be hydrolyzed to produce genipin which reacts readily with taurine to produce an attractive blue color. Four greens, two blues and one red colorant have been commercialized in Japan. They have been suggested for use with candies, condiments, ices, noodles, imitation crab, fish eggs, chestnuts, beans, dried fish substitutes, liqueurs, baked goods, etc.



### 12.15.3 Toxicology

The geniposides from gardenia were found to have some hepatotoxicity due to the aglycone genipin produced by hydrolysis of the geniposides.<sup>34</sup> The yellow, green, red, and blue colorants were studied extensively and were found to be safe for human consumption as food colorants.<sup>35,36</sup>

### 12.15.4 Future prospects

The yellow crocins from gardenia have received some success for the same colorant applications as saffron,<sup>37</sup> but the iridoid derivatives have not received the same promotion. The range of colorants available from the same source would seem to make them attractive possibilities.

## 12.16 Phycobilins

### 12.16.1 Introduction

The phycobilins belong to the heme group of pigments which include the green chlorophylls in plants and the red hemoglobins in animals. The phycobilins are major biochemical components of the blue-green, red, and cryptomonad algae.<sup>38</sup>

### 12.16.2 Chemistry and usage

Phycobilins are colored, fluorescent, water-soluble pigment-protein complexes. They can be classified into three groups according to color: phycoerythrins are red with a bright orange fluorescence; phycocyanins and allophycocyanins are both blue and fluoresce red. The structures of two are shown in Fig. 12.3. Phycocyanins and allophycocyanins share the same chromophore but differ in the protein portion. The attachment of the bilin chromophore to the protein is very stable and this makes them desirable from a colorant point of view. Phycobilin preparations can be made by simply freeze drying algal cell suspensions which can be grown in ponds or sophisticated tubular reactors. Suggested applications involve chewing gums, frozen confections, dairy products, soft drinks, and ice cream.

### 12.16.3 Toxicology

One study on toxicity of phycocyanin from *Spiroulina platensis* reported no adverse effects.<sup>39</sup> There is little other data available on toxicology but no toxic effects would be expected in view of the long history of algal consumption.

### 12.16.4 Future prospects

The future of the phycobilins looks promising for two reasons. First, there are no other blue natural colorants available and, admittedly, blue is not a favorite food

color but niche markets are evolving. Second, spirulina is becoming an attractive product in the health food area, and the same facilities used to produce spirulina products could be used to produce phycobilins.

## 12.17 Caramel

### 12.17.1 Introduction

Caramel is a brown colorant obtained by heating sugars.<sup>33</sup> The official FDA definition is as follows: 'The color additive caramel is the dark brown liquid or solid resulting from carefully controlled heat treatment of the following food grade carbohydrates: dextrose, invert sugar, lactose, malt syrup, molasses, starch hydrolysates and fractions thereof, or sucrose.' Heating sugar preparations to produce brown flavorful and pleasant-smelling products has been practised in home cooking for centuries but the first commercial caramel colorants appeared in Europe about 1850.

### 12.17.2 Chemistry and usage

Commercial caramel is a very complex mixture of heat degraded carbohydrates. In 1980, the JECFA recommended that further information on the chemical properties be obtained in order to establish a suitable classification and specification system. The International Technical Caramel Committee attempted to provide this information and undertook an extensive research program.

The complexity of the mixtures made it impossible to define the chemical composition so the commercial preparations were divided into four groups (Table 12.2) on the basis of a series of sophisticated chemical assay procedures. Caramel colorants must be compatible with the food products in which they are used, which usually means the absence of flocculation and precipitation in the food. These undesirable effects result from charged macromolecular components of caramel which react with the food. Hence the net ionic charge of the caramel macromolecules at the pH of the intended food product is the prime determinant of compatibility. Caramel colorants are used in a variety of foods (Table 12.2) but over 80% of the caramel produced in the US is used to color soft drinks particularly colas and root beers.

### 12.17.3 Toxicology

One of the major considerations of the research requested by JECFA was the safety aspects which was not surprising in view of the chemical complexity of the caramels. The program resulted in the publication of 11 papers in the same issue of the journal *Food and Chemical Toxicology* 1992 (Vol. 30) and seven of them were on toxicology. Caramel colorants were given a clean bill of health and JECFA assigned an ADI of 0–200 mg/kg/day.

**Table 12.2** Caramel formulations

Class	Charge	Reactants	Usage
1	—	No ammonium or sulphite compounds	Distilled spirits, desserts, spice blends
2	—	Sulphite compounds	Liqueurs
3	+	Ammonium compounds	Baked goods, beer, gravies
4	+	Both sulphite and ammonium compounds	Soft drinks, pet foods, soups

### 12.17.4 Future prospects

Caramel colorants are well established in food formulations and probably will remain that way in the foreseeable future.

## 12.18 Brown polyphenols

### 12.18.1 Introduction

There are two important sources of brown polyphenols, cocoa and tea, used as colorants for foods. Both are very old and date back to antiquity.

### 12.18.2 Chemistry and usage

The cacao plant, *Theobroma cacao*, is the source of chocolate which is well known and highly prized in international commerce. The cacao pods contain beans which are fermented and pressed to provide a brown liquid which is the raw material for chocolate. The press cake is ground and sold as cocoa and it also provides a brown colorant. The pods, beans, shells, husks and stems have also been suggested as colorants. They contain a very complex mixture of acyl acids, leucoanthocyanins, flavonoid polymers, tannins, and catechin-type polymers.<sup>33</sup>

The tea plant, *Thea sinensis*, has provided a desirable beverage for centuries but it is also used as a colorant. Extracts of tea contain a very complex mixture of glycosides of myricetin, quercitin and kaempferol, epicatechin, epigallocatechins, acyl acids and many other polyphenol compounds.<sup>33</sup> In black tea, the above compounds may act as precursors to the poorly defined compounds thearubin and theaflavin.

Both cocoa and tea are used in a variety of food products, including beverages, bakery products, confections, toppings, dry mixes, etc.

### 12.18.3 Toxicology

Tarka<sup>40</sup> wrote an extensive review of the toxicology of cocoa and the methylxanthenes, theobromine, caffeine and theophyllin. The review involved biological and behavioral effects, metabolism, species variation, pregnancy,

lactation, reproduction, teratology, mutagenicity, fibrocystic breast diseases, and drug and dietary factors. No implications for human use as a beverage or a colorant were discussed. The toxicology of tea is similar to cocoa and no adverse effects for humans have been implied.

#### **12.18.4 Future prospects**

Both cocoa and tea are well entrenched and this is not likely to change. Neither group is permitted as a food colorant in the US but they are permitted as food ingredients and this accomplishes the same end.

### **12.19 Titanium dioxide**

#### **12.19.1 Introduction**

Titanium dioxide is a large industrial commodity with world production over 4,000,000 tons but only a very small proportion is used as a food colorant. Commercial  $\text{TiO}_2$  is produced from the mineral ilmenite, which occurs in three crystalline forms, but the only one approved for food use is synthetic anatase. Anatase occurs in nature but only the synthetic version is approved because it contains fewer impurities.<sup>41</sup>

#### **12.19.2 Chemistry and usage**

Titanium dioxide is a very stable compound with excellent stability towards light, oxidation, pH changes, and microbiological attack. It is virtually insoluble in all common solvents. It is available in oil-dispersible and water-dispersible forms with a wide variety of carriers. Titanium dioxide is a very effective whitener for confectionery, baked goods, cheeses, icings, toppings, and numerous pharmaceuticals and cosmetics.

#### **12.19.3 Toxicology**

Titanium dioxide has been subjected to a number of safety tests<sup>11</sup> and found to be non-genotoxic, non-carcinogenic and exhibited no adverse effects in rats, mice, dogs, cats, guinea pigs, and rabbits. The  $\text{LD}_{50}$  values are greater than 25 g/kg/day for rats and 10 g for mice. Apparently titanium dioxide is poorly absorbed and non-toxic. JECFA has not established an ADI since they consider titanium dioxide to be self regulating under GMP. In the US, it is allowed up to 1% by weight in food.

#### **12.19.4 Future prospects**

Titanium dioxide is well established and will probably remain that way.

## 12.20 Carbon black

### 12.20.1 Introduction

Carbon black is a large volume industrial commodity but its food use is very small.<sup>40</sup>

### 12.20.2 Chemistry and usage

Carbon black is derived from vegetable material, usually peat, by complete combustion to residual carbon. The particle size is very small, usually less than 5  $\mu\text{m}$ , and consequently is very difficult to handle. It is usually sold to the food industry in the form of a viscous paste in a glucose syrup. Carbon black is very stable and technologically a very effective colorant. It is widely used in Europe and other countries in confectionery.

### 12.20.3 Toxicology

In the US in the 1970s when the GRAS list was being reviewed, safety data were requested on carbon black in view of the possibility that it might contain heterocyclic amines. Apparently, the cost of obtaining the data was higher than the entire annual sales of food grade carbon black so the tests were never done. Carbon black is not permitted in the US.

## 12.21 Miscellaneous colorants

A number of preparations are used in small volume or with minimal effect on color.<sup>41</sup> Ultramarine blue, a synthetic aluminosulphosilicate blue colorant, is widely used in cosmetics and in salt intended for animal consumption. A variety of brown iron oxides are used in cosmetics, drugs and pet foods. Talc is a large industrial commodity with many uses. It is used as a release agent in the pharmaceutical and baking industries as well as a coating for rice grains. Zinc oxide is an effective whitener for food and food wrappers. It is also added as a nutrient. Riboflavin provides an attractive yellow green color to foods as well as a nutrient benefit. Corn endosperm oil is added to chicken feed to enhance the yellow color of the skin and eggs. Dried algal meal is produced from *Spongiococcus spp.* and may be added to chicken feed to enhance the color of skin and eggs. Extracts from other algae are permitted in other countries. Four products from cottonseeds may be added to food but they are usually considered ingredients and impart only a slight yellow color. Shellac, obtained from the insect, *Lassifer lacca*, is added to foods as a surface coating or glaze and it does not affect the appearance. Octopus and squid ink contain mixtures of meloidin polymers and are effective black colorants for pasta for special occasions for some ethnic groups. They are not permitted in the US. All of the above preparations are considered harmless for human consumption by virtue of a long

history of use and sometimes because their use is in such small amounts or concentrations as to be considered of no concern even though the toxicological data may be a little hazy.

## 12.22 Outlook

Colorants present a wide variety of preparations added to foods to increase their visual appeal. Depending on their classification, they may be subjected to extensive or minimal toxicity testing. For example, FD&C Red No. 2 is probably the most tested additive in our food supply, second only to saccharin. Others have been grandfathered in by virtue of a long history of consumption. But our society is moving towards a more formulated food supply with more attention being paid to ingredients available in large quantities but not necessarily in the most appealing form. Colorants and other additives, such as flavorants and texturants, are vital to making our food supply appealing. Interest in colorants, in particular, is increasing as judged by the research activity. Over the last 50 years, there has been a distinct trend towards the use of natural colorants as compared to synthetic 'coal tar dyes'. In one study,<sup>21</sup> the five-year period from 1979–1984 yielded the same number of colorant patents as the previous ten-year span from 1969–1978. In the fifteen-year span, there were 356 patents on natural colorants and 71 on synthetics. This trend has continued and shows no sign of changing.<sup>42–47</sup> The confidence in natural colorants over synthetics may be a little naive but it is realistic.

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# 13

## Developments in natural colourings

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### 13.1 Introduction: the use of natural colourings in food

Over the past decade the use of natural colours in food and beverages has increased at a much greater pace than that of synthetic colours. There are a number of reasons for this development, which is based on both technological improvements as well as market trends. This chapter introduces the natural colours that are applied by the food and beverage industries, describing the whole range from yellow through orange, red and green to brown and black. It explains the many factors that are important to be aware of in their use during selection, quality control, storage and processing. The recent developments in technology that have helped promote the increased use of natural colours are explained with special focus on microencapsulation. Also the use of antioxidants and the advantages of emulsions and suspensions to expand the use of certain pigments are described. Finally there are suggestions on how present trends will affect the future use of natural colours.

The chapter's focus is the application of natural colours in food and beverage products, as this is where the primary challenge lies. By understanding the many factors that influence the use of natural colours, it is possible to achieve colouring solutions that are bright and intense as well as stable under processing and during the food or beverage product's shelf life.

### 13.2 The range of natural colourings<sup>1</sup>

The range of natural colourings goes from yellow through orange, red and green to brown and black. In many cases different raw materials or blends of these can

be used to achieve the same or similar shades, whereas others are very distinct. In the following section, each of the major natural colours will be described briefly with regard to raw material, processing, typical end products, applications and critical parameters.

### 13.2.1 Turmeric

The turmeric plant, *Curcuma longa*, is cultivated in many tropical countries, with the primary commercial production taking place in India. It has been used as a spice for thousands of years and is one of the principal ingredients in curry. The main colouring pigment is curcumin. The turmeric roots are harvested around February, ground to a powder and subsequently extracted with solvent. The resulting turmeric oleoresin has a curcumin content of 37 to 55% and the same relative proportion of flavour compounds and colour as in the spice.

The pure colour is produced by further crystallisation of the oleoresin resulting in a product with minimum 90% curcumin and very little of the flavour compounds. It is insoluble in water and poorly soluble in other solvents. Typical products for use in the food industry have a curcumin content of 4 to 10% and are achieved by dispersing pure curcumin in a mixture of food-grade solvent and emulsifier, by dissolving in vegetable oil or by spraying onto starch.

Curcumin in aqueous media has a lemon-yellow colour with a distinct green shade at low pH. Typical applications include dairy products, sugar confectionery, ice cream, water ice, bakery and savoury products. Curcumin has good heat and acid stability, but is sensitive to light. It can, however, be used in products packed in non-transparent packaging.

### 13.2.2 Annatto

Annatto is the seed of the bush, *Bixa orellana*, which is mainly found in Central and South America and in East Africa. Traditionally, annatto seeds are also used as a spice, often blended with other ingredients before addition to soups and meat dishes. The seeds grow in large clusters of capsular fruits that upon harvesting are dried in the sun, cracked open and taken out by hand. The basic colour pigment is bixin, a natural carotenoid that is found in the thin resinous coating of the seed. It can be extracted in different ways to yield oil-soluble extracts, oil suspensions or water-soluble extracts.

Oil-soluble extracts are made by extraction of annatto seeds with hot vegetable oil, resulting in products with low colour content, typically 0.1–0.2% bixin, as bixin has poor solubility in oil. By suspending the undissolved colour pigment in vegetable oil or fat base the content of bixin can be increased by up to 5%. Oil-soluble extracts and oil suspensions are yellow-orange and suitable in products with a high quantity of oil present, i.e., margarine and butter, salad dressings and extruded snacks.

By extracting the annatto seeds with water and potassium hydroxide, bixin is converted into the water-soluble pigment norbixin. Water-soluble solutions

typically contain 0.5–4% norbixin, and powders with concentrations as high as 15% can be achieved through further spray-drying. Bixin and norbixin can also be blended to produce colours applicable in both water and oil-based foods. Typical applications for norbixin products are cheese, ice cream and water ice, bakery, sugar confectionery and beverages, where they give a yellow-orange colour. Both norbixin and bixin are reasonably stable to heat, whereas light stability is best if norbixin is bound to protein, i.e., in cheese. Norbixin may precipitate at low pH and cannot be mixed with products containing calcium. However, it is possible to formulate norbixin products that are stable at low pH.

### 13.2.3 Paprika

The source of paprika is found in the pods of the sweet red pepper, *Capsicum annuum*, which is grown in Spain, Central Europe, USA, Asia and Africa. It is used by the food industry for both colouring and flavouring purposes, giving an orange-red colour and a characteristic taste. Paprika consists of several different carotenoid pigments that are developed during ripening and are responsible for its red colour. The most important are capsanthin, capsorubin and beta-carotene, accounting for about 90% of the total pigments. The strength is expressed as colour units (C.U.) per g. For use as a colour, paprika oleoresin is obtained by solvent extraction of the pods. The extract is standardised with vegetable oil to the desired colour strength, the most common being 40,000, 60,000, 80,000 and 100,000 C.U. These extracts can be further processed to obtain water-miscible products, typically by incorporating an emulsifier during production.

Paprika has a distinctive flavour and is therefore typically used in savoury products such as meat, soups, sauces and snacks. Deflavoured products are available for use in sugar confectionery and beverages, but so far they are not very widespread. Stability of paprika products to heat and light is fairly good and can be improved considerably through stabilisation with antioxidants. Figure 13.1 shows the most common raw materials for the yellow to orange colours.

### 13.2.4 Other carotenoids

Carotenoids are very widespread in nature, accounting for many yellow, orange and red colours. Most fruits and vegetables contain mixtures of carotenoids, and more than 600 different types have been identified. Besides annatto and paprika, commercial colouring of food and beverages primarily uses mixed carotenoids, beta-carotene and lutein. Nature's richest source of mixed carotenoids is the fruit of the oil palm tree, *Elaeis guineensis*. Predominantly grown in Malaysia and Indonesia, the oil palm tree contains a mixture of beta- and alpha-carotene with traces of gamma-carotene and lycopene. The fruits are first processed to crude palm oil and further steps include distillation, purification by HPLC and



**Fig. 13.1** Yellow to orange raw material sources; turmeric finger, paprika pods and annatto capsules.

standardisation to an oil suspension from which oil- and water-soluble products in different strengths can be made. The oil-soluble products give a fresh yellow colour shade in application whereas the water-soluble emulsions can range from yellow to yellow-orange.

Beta-carotene is the most common of the single carotenoids and is used extensively in the food and beverage industries, giving a yellow-orange colour in application. Most beta-carotene applied today is manufactured by synthesis resulting in a molecule equivalent to that found in nature. However, several natural sources are available and are increasingly used to replace the synthetic variant. Beta-carotene produced from the micro algae, *Dunaliella salina*, combines photosynthesis with a microbial fermentation and takes place in open ponds in Australia, Israel and USA. The algae are grown in 30% salt concentration with high light intensity, high temperature and with or without mechanical agitation. Under these conditions the algae produce large amounts of carotenoids and can accumulate beta-carotene up to 10% of their dry weight. Beta-carotene is purified from the dried algae by a series of processing steps including concentration, enzyme treatment, extraction, saponification, partial crystallisation and dispersion in oil at a concentration of 20–30%.<sup>2</sup> Beta-carotene can also be obtained from a fermentation process with the mould, *Blakeslea trispora*. Two types of the fungus are mated in a specific ratio and large amounts of beta-carotene are synthesised in aerobic submerged batch fermentation. Beta-carotene is subsequently recovered by solvent extraction, purification, concentration and crystallisation to obtain either a crystalline beta-carotene with purity greater than 96% or a 30% micronised suspension in oil.<sup>3</sup>

Both mixed carotenoids and beta-carotene are available as oil- and water-soluble products in a number of different strengths. They are technically suitable in a wide range of applications such as beverages, sugar confectionery, ice

cream and water ice, dairy products, fruit preparations, bakery and spreads and have good stability towards heat, light and pH. Their application is most often limited by their price.

Lutein is the solvent extract of dried flower petals of marigold, *Tagetes erecta*, which has been further concentrated and deodorised. It can also be derived from alfalfa grass as a by-product of chlorophyll extraction. Following extraction it can either be suspended in vegetable oil or made water dispersible by the addition of emulsifier. Lutein gives a warm yellow colour in application, but is not widely used in food. It has advantages over turmeric in, for example, beverages and sugar confectionery when good light stability is required. Besides their use as a colour, carotenoids also have nutritive properties and are therefore also used as active ingredients in health products.

### 13.2.5 Carmine/cochineal extract

Both carmine and cochineal extract are made from the dried female insect, *Dactylopius coccus costa*, commonly known as cochineal. The cochineal live on specific cacti and the main production is in Peru, but cochineal are also produced in Chile, Bolivia and the Canary Islands. There are usually two harvests per year, where the cochineal are removed manually with suitable tools from leaves of the cacti. Following harvest the insects are sun dried before further processing. The cochineal insects are extracted using an aqueous alkaline solution. The resulting cochineal extract can either be formulated to different cochineal products in powder or liquid form or further processed to carmine. Cochineal products are water-soluble and orange in low pH applications. The colour intensity is relatively low.

Carmine is achieved by the complexing of cochineal extract with calcium and aluminium. It can be standardised with maltodextrin or lactose and sold as carmine lake products of different concentrations and different shades of pink through red and violet to blue. Carmine is insoluble in water and acids, but soluble in alkali. By dissolving carmine lake in aqueous potassium, sodium and ammonium hydroxide, water-soluble powders and solutions can be made. Carmine has high colour intensity and is therefore more cost-effective than cochineal extract.

Carminic acid is the active pigment in both cochineal extract and carmine. Cochineal extracts are typically sold as powders with a high concentration of carminic acid or as low concentration liquids. Carmine lakes and water-soluble carmine powders typically have concentrations from 30 to 60% carminic acid, whereas carmine solutions have low concentrations of 2 to 10%. The main applications are meat, sugar confectionery, beverages, ice cream, dairy products and cosmetics. The only significant technical limitation on the use of carmine is low pH, where precipitation can occur at pH below 5. Acid stable carmine has been developed to overcome this limitation, giving a red shade at low pH. Carmine and cochineal extract are very stable to heat and light and resistant to oxidation. Cations will affect the colour shade, generally increasing the blueness of the colour.

### 13.2.6 Anthocyanins

Anthocyanins are a group of natural polyphenolic pigments responsible for the red to blue colour of a wide range of fruits and vegetables. Traditionally the most common sources of industrially used anthocyanins have been grapes, elderberry and blackcurrant, but the past years have also seen new sources launched in the market such as red cabbage and black carrot, Fig. 13.2. Due to an intramolecular copigmentation of their phenolic structure they are more stable to light, pH and heat than traditional sources. The fruits and vegetables used for anthocyanin production come from cultures with a yearly crop and are primarily grown in Europe.

The different raw materials are extracted with water, concentrated, standardised and pasteurised. Subsequently they can be spray-dried to powder formulations using maltodextrin as a carrier. Anthocyanins are available in different strengths from various sources and in both liquid and powder formulations. The main food applications are beverages, fruit preparations, sugar confectionery and water ice. All anthocyanins are very pH dependent and they change colour hue from red at low pH through blue to brownish-green at high pH. Their colour intensity is also reduced with increasing pH and they are most commonly used in low pH applications. Stability to heat and light is generally good, but varies with the source. Anthocyanins also have nutritive properties and are used as active ingredients in health products.



**Fig. 13.2** Different anthocyanin sources; grapes, black carrots and red cabbage.

### 13.2.7 Red beet

Red beet colour is obtained from the beetroot vegetable, *Beta vulgaris*, which has been cultivated for centuries in temperate climates. The pigments present in beetroot consist of a group of red pigments and a group of yellow pigments. In most varieties of beetroot, the red pigment betanin is the predominant colouring compound, representing 75 to 90% of the total colour present. Beetroot is harvested in autumn and the juice is extracted by physical means, often a press operation under acidic conditions. It is further concentrated by ultra filtration and pasteurised to yield a viscous liquid with approximately 0.5% betanin. This juice can further be spray-dried to a powder with a betanin content of approx. 0.35% and using maltodextrin as a carrier.

Red beet gives a nice strawberry colour in ice cream, dairy products, fruit preps, jams and jellies as well as sugar confectionery products, which are not subjected to heavy heat treatment. It is a very intense colour and dosage levels are usually low. However, the pigment is susceptible to heat degradation and oxidation, which limits its use but can, in part, be overcome by adding the colour after heat treatment. Stability is highest at pH 4.5, and red beet colour is not recommended for alkaline applications.

### 13.2.8 Chlorophyll

Chlorophyll is present in all plants capable of photosynthesis. The usual sources for colour production are grass, spinach or alfalfa and chlorophyll is extracted from the dried plants by solvent extraction. During the extraction process a magnesium ion is wholly or partly lost from the pigment, making it more unstable and resulting in a product that is duller in appearance and of an olive-green colour. This chlorophyll extract is oil-soluble and can be further standardised with vegetable oil or blended with a food solvent or emulsifier to produce a water-miscible form. The products are sold as uncoppered chlorophyll.

The magnesium ion, which is lost during the extraction process, can be replaced with copper to produce a more stable complex with a higher tinctorial strength. This complex, copper chlorophyllin, is made by further processing of chlorophyll extract with water and alkali addition. Chlorophyll is recommended where a green colour is required in bakery products, dairy products, sugar confectionery, cereals, jams and jellies. Chlorophyll is also used as a complementary colour, when decolourisation of yellowish cheese milk is needed. Chlorophylls are most stable under alkaline conditions and in diluted acids they will lose colour rapidly and copper chlorophyllins will precipitate. Copper chlorophyllins are relatively heat and light stable.

### 13.2.9 Caramel

Caramels are obtained through controlled heat treatment of food-grade carbohydrates, i.e., dextrose, glucose or saccharose. The caramelisation process



**Table 13.1** Different types of caramel

	Type 1	Type 2	Type 3	Type 4
Name	Plain	Caustic sulphite caramel	Ammonia caramel	Sulphite caramel
Manufactured SO <sub>2</sub>	—	+	—	+
with NH <sub>3</sub>	—	—	+	+

consists of reducing large carbohydrate molecules to smaller carbohydrates under the effect of heat, pressure and acidity. There are four different caramel types, which are distinguished by their production method, see Table 13.1. Approximately 90% of the world use of caramel is ammonia or sulphite caramel (types 3 and 4). Caramel colours are available both as liquids and powders with varying colour strengths. Typical applications together with stability towards different parameters are given in Table 13.2.

### 13.2.10 Carbo vegetabilis

Carbo vegetabilis, or carbon black, is the only natural colour providing shades within the grey to black area. It is produced from vegetable material, usually peat, and processed to insoluble carbon by combustion and milled to a very fine powder with particle sizes less than 5 µm. As this powder is very difficult to handle, it is suspended in glucose syrup and sold as a viscous paste. Carbo vegetabilis is primarily used in sugar confectionery, ice cream and water ice. A pure black colour can be achieved with high doses, whereas lower doses provide various shades of grey. The pigment is very stable towards light, pH, heat and oxidation.

**Table 13.2** Caramel applications

	Type 1	Type 2	Type 3	Type 4
Alcohol stability	High	High	Poor	Moderate
Protein stability	Low	Low	High	Low
Acid stability	Low	Moderate	Low	High
Tannin resistance	Low	High	Low	High
Colour intensity	Low	Low	Moderate	High
Viscosity	High	High	Moderate	Low
Main applications	Whiskey and other spirits	Vermouth, brandy and other alcoholic drinks with high tannin levels	Beer, vinegar, sauces, baking and confectionery	Soft drinks

### 13.2.11 Other natural colours and colouring substances

The natural colours described above are those that are most well known and most widely used by the food and beverage industries. Other colours include saffron, gardenia, carthamus, lycopene and monascus. Besides these, a number of substances are added to food and beverages for other purposes than to colour, but with a secondary colouring effect. This increases the list of available natural colouring substances considerably. The use of natural colours is subject to different legislation around the world and local regulation should always be consulted before choosing a final solution.

## 13.3 Factors in selecting natural colours

From a technological perspective the use of natural colours is more complex than that of synthetics. However, by being aware of the different critical parameters it is possible to apply natural colours with good results in most food and beverage products. Natural colours are used in a wide number of different food and beverage products and hence it can be difficult to outline general guidelines for their use. However, some parameters should be considered before choosing a colour in order to find the most suitable solution in a given application.

Initially, the shade and intensity of the colour solution has to match the target group as well as the consumer's perception of the flavour. Children tend to be attracted to bright and vivid colours, whereas adults prefer shades that are more subdued. There are also large geographical variations in the perception of colours related to a specific flavour, and thus a strawberry colour may differ from orange-red to bright pink. The chosen colour should also match the colour of the relevant packaging and any other products within the same brand. A number of parameters are discussed below and an overview of these is seen in Table 13.3.

### *Food matrix*

The food matrix will indicate whether to use oil- or water-soluble colours. In emulsions that have both oil and water phase, it will generally be most economical to colour the continuous phase, i.e., use oil-soluble and suspendable colours in margarine and cream fillings, and water-soluble colours in milk drinks, dressings, etc. However, in products with a high content of the dispersed phase, i.e., mayonnaise and low fat spreads, a deviation from the general rule of thumb can be an advantage from a cost point of view.

### *Recipe*

Some ingredients such as sugar and protein have a tendency to stabilise most natural colours, whereas others, i.e., salts, some colloids, ethanol and sometimes flavours, may have a negative influence on the stability of the added colour. High amounts of ascorbic acid, sulphur dioxide and other antioxidants may

**Table 13.3** Factors that influence the choice of natural colours

In the application	Colour characteristics
Food matrix	Oil- or water-soluble colours
Recipe	Colour stability
pH	Acid stable colours
	Colour shade changes
Background colour	Colour dosage
Heating	Heat stable colours
Other processing steps	Colour dosage
Size	Colour dosage
Packaging material	Light stable colours
Religion, specific ingredients	Kosher, halal, GMO, vegetarian
Legislation	Approved by local legislation
Price	Cost in use

result in degradation of colours like anthocyanins. In emulsions and suspensions the intensity of a colour will vary with the amount of dispersed phase, the particle size and the content of dry non-fat solids. For example, a water-soluble pigment in a low-fat milk drink will look much brighter and more intense compared to the same dosage in a milk product with a higher fat content.

### *pH*

Acid stability of the colour is required in low pH applications like soft drinks and many confectionery products. Alkaline formulations of chlorophyll and annatto colours are the most sensitive, and acid-proof formulations should be preferred. However, increased acid stability may be achieved if the annatto pigment norbixin is able to bind to protein, e.g., fruit pulp in fruit based beverages.

Alkaline carmine formulations can be used in acid conditions if the colour is entrapped in the viscous food matrix. Hence water-soluble carmine has excellent stability in ice-lollies, hard-boiled candy, gums, jellies and the like. If used in a non-viscous product, e.g., soft drinks, the aluminium bond in the carmine may break and the colour will precipitate thereby changing hue towards a more orange shade.

Some colours, like all the different anthocyanins, change colour shade with varying pH. At pH around 3, where they are most commonly used, they will display bright pink and reddish hues. Moving towards neutral pH the colour will change through purplish-red to mauve and bluish shades and, depending on the anthocyanin source, can even appear dull and dark.

### *Background colour*

Background colour can influence the colour of the final food or beverage. The background colour can come either from other ingredients, e.g., fruit juices, spices and dairy fat, or be generated during production, e.g., Maillard reaction

products. It may be necessary to mask an off-colour and it can be quite a challenge to colour a greyish or brownish base bright and clear compared to starting from a pure white background. The choice of colour can also be influenced by the clarity of the background. Colours that look very similar and bright in a clear matrix may appear totally different in a white base, where one can appear dull and another may look vivid and appealing.

### *Processing*

Processing parameters are also essential to consider, the most critical one normally being heating. Not all natural colours are equally heat stable. Colours like carmine and turmeric are generally considered to have excellent heat stability and can be used in, for example, UHT products. Red beet shows poor heat stability and tends to turn brownish during heating. The actual food matrix may also influence the heat stability.

Aeration, as seen in ice cream, desserts, some confectionery products and expanded crisps for example, will dilute colours thereby lowering their intensity and increasing their lightness, and a higher dosage of colour might be needed. For some applications it is also important that the colour does not contain emulsifier as this can reduce the expansion and influence stability of the incorporated air cells.

### *Size*

When recommending dosages it is also important to consider the actual size of transparent and semi-transparent food products. The distance the light is transmitted influences the intensity of the colour, and therefore a bigger jelly will look darker and more intense compared to a small one, even with the same recipe. Likewise a soft drink in a small bottle will appear lighter than the same drink in a larger bottle.

### *Packaging material*

Light transparent packaging material will normally require light stable colours. Conventional turmeric formulations and red beet are the most light-sensitive natural colours, but low water activity tends to stabilise especially turmeric and thereby delay fading. However, due to technological developments, light stable natural colour products including turmeric are now commercially available.

### *Religion*

In many countries around the world religious requirements also influence the choice of natural colour product. To match consumer demands, colours must often be accompanied by kosher or halal documentation, which can include certification of both raw materials and processing.

### *Specific ingredients*

A number of recent food scares also play a role in the choice of natural colour product. Many food and beverage manufacturers are avoiding the use

of genetically modified ingredients and bovine material to overcome consumer insecurity related to gene technology, foot and mouth disease and BSE. Vegetarian requirements also affect the choice of ingredients that can be used in natural colours and certification of the above issues is often a demand.

### *Legislation*

Not only functional properties of the colour must be considered but also local legislation should be taken into account. The EU Directive 94/36/EC, often referred to as the Colours Directive, is implemented throughout the member states.<sup>4</sup> Here the approved colours are listed together with conditions for their use. Several non-EU countries such as Norway and Turkey have adopted this directive and some Eastern European countries are also preparing to follow it, as their membership of the EU moves closer.

In the US the use of colours is outlined in Code of Federal Regulations, Title 21 (21 CFR).<sup>5</sup> Colours are divided into two groups: certified colours and colours exempt from certification. Natural colours are included in the last group, but the list differs from those colours permitted in the EU. A number of countries also follow the US legislation. The Australian<sup>6</sup> and Japanese<sup>7</sup> legislations on colours in food are also used as the basis for local regulation in a number of other Asian countries. Legislation changes regularly and monitoring of relevant local requirements is recommended.

### *Price*

Compared to other expenses such as raw materials, processing, packaging, marketing and distribution, the price of colours is small in most food and beverage products. However, the cost in use of different natural colours is still a very important factor. Generally, yellow colours like turmeric and annatto are economical to use, whereas synthetic beta-carotene, natural beta-carotene and mixed carotenoids are rather expensive in application. Within the natural red shades red beet is normally inexpensive but also has limitations with regards to functionality. The bright and stable colours originating from carmine and different anthocyanins are more expensive in use.

## **13.4 Quality control issues**

When receiving natural colour products from the supplier it is normal to carry out a quality control. The extent of this control will typically depend on the type of colour product, the information available on the certificate of analysis, the expected processing conditions, the finished product and the standard quality control procedures of the food or beverage manufacturer. In some cases the colour strength or colour hue and intensity may be the most relevant parameter. In others the microbiological standard and absence of pathogens is also important. For powder formulations to be used in dry blends, the particle size is

relevant, whereas density can be central if the colour is volumetrically measured by, e.g., flow meter during the production process.

### **13.5 Storage and handling issues**

Most colour products are recommended cool storage (4–8°C) to minimise both the risk of microbiological growth and the degradation of colour pigments. Colour formulations containing sugar, protein or relatively high amounts of water as, for instance, many emulsions and liquid extracts of beetroot, elderberry, etc., will be sensitive to microbiological hazards. Other products which have low colour strengths in general have shorter shelf lives due to degradation of pigments. Paprika, however, should be stored at ambient temperature to avoid separation due to coagulation of different components at low temperature. Many colours in powder form are hygroscopic and must be protected from humid air to avoid clotting.

In general it is important to follow the storage guidelines from the supplier to ensure stability during the shelf-life period and an optimal performance of the natural colour during later use.

Besides the many factors to be aware of when selecting natural colour products as described in Section 13.3, the handling during production is also important. Good guidance on how to handle colours will normally be provided by the supplier in the safety data sheet specifying the directions of use as well as any relevant hazards.

When handling colours during processing the risk of contamination of especially the more sensitive colours can be minimised if it takes place in clean environments. The primary hazard related to natural colours is the possible irritation of skin and eyes when using alkaline formulations.

### **13.6 Improving natural colour functionality**

Many food and beverage manufacturers are still reluctant to use natural colours due to former issues such as dull colour shades, poor stability with rapid fading and difficulties in handling. However, over the past ten years natural colour manufacturers have discovered new techniques within formulation and processing that are able to resolve these issues. Combined with the use of high-quality raw materials it is now possible to develop and manufacture products that have better functional properties both during handling and in the final food products.

Most efforts have been put into developing natural colours with improved stability towards low pH, light and heat and with increased brightness of the different pigments. The technologies that have been applied are micro-encapsulation, addition of antioxidants, emulsions and oil suspensions.

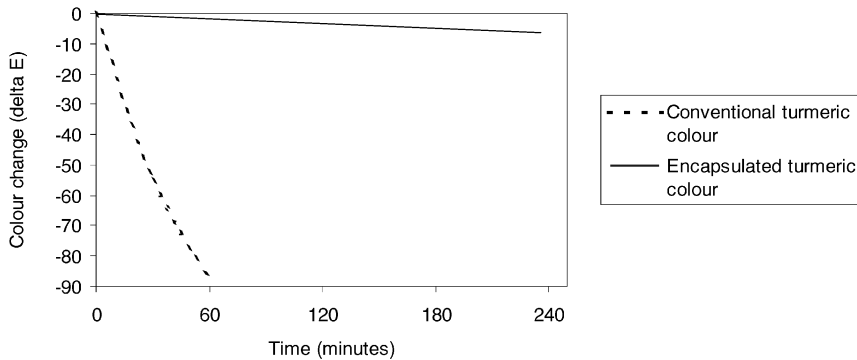
### 13.6.1 Microencapsulation

Microencapsulation of different substances is a very popular technique that is being increasingly applied in a variety of industries. In the pharmaceutical industry it is used to ensure transportation of drugs through the digestive system until the optimal time of uptake. For flavours, microencapsulation can be used to improve their overall stability as well as delaying their release until the optimal time during cooking or eating. In the foregoing examples it is imperative that the microencapsulation is broken down at a given time or under a given circumstance. For colours, however, the challenge is to maintain the protection given by the coating despite the influence of different chemical and/or physical factors in the final food and beverage products or during their manufacture. As the majority of food and beverage products are water-based, microencapsulation of colours is especially beneficial for pigments, which are not soluble in water. Examples of these are various carotenoids such as beta-carotene, lutein, annatto and paprika as well as turmeric, carmine, chlorophyll and carbo vegetabilis.

A number of different microencapsulated colours are available that vary with regard to coating, production method and final formulation. The coating can be created with diverse food ingredients such as pectins, gums and carbohydrates, but also proteins and lipids may be used. Several different production methods for microencapsulated colours have been published,<sup>8,9,10</sup> which include processing steps such as blending, comminuting, standardisation and drying. A high and specified quality of raw material must be assured, and it is also important to monitor colour shade and functionality at critical control points both during manufacture of the microencapsulated colour and in the final product. The microencapsulated colour may be available in both liquid and powder form.

There are a number of advantages that can be achieved with microencapsulation of colours. Some of these are that the colours are easier to handle, weigh out and add into food systems. The higher water dispersibility also allows fast and efficient cleaning of processing equipment. Control of particle size during the encapsulation process is essential to ensure optimal use of the colour pigments. The colouring effect is a result of light reflection from the particle surface, and thus there is a close correlation between the total particle surface and the colour intensity. The particle size distribution is also of great importance for the perceived colour stability in liquid products such as beverages, as larger colour particles have a tendency to sediment.

Carmine and curcumin are two of the colours where major advantages can be achieved by microencapsulation. For carmine, stability under acid conditions is greatly improved and it is possible to use this colour in beverages to achieve a pink, cloudy appearance. In standard formulations curcumin normally shows rather poor stability when exposed to light but by entrapping it in appropriate encapsulation material it is possible to achieve excellent light stability and a bright lemon colour (Fig. 13.3). This is especially appreciated in sugar confectionery products, soft drinks and dressings when they are packed in



**Fig. 13.3** Conventional and encapsulated turmeric colour dissolved in a soft drink media (30 mg curcumin/litre soft drink media (10% sucrose, pH3)) and exposed to light in a Heraeus accelerated lighting unit (480 W/m<sup>2</sup>, 300–800 nm). Colour change is measured using a Minolta Tristimulus CT310 calibrated with demineralised water.

transparent packaging. An increased light stability is also achieved with encapsulation of other colours, e.g., annatto and paprika.

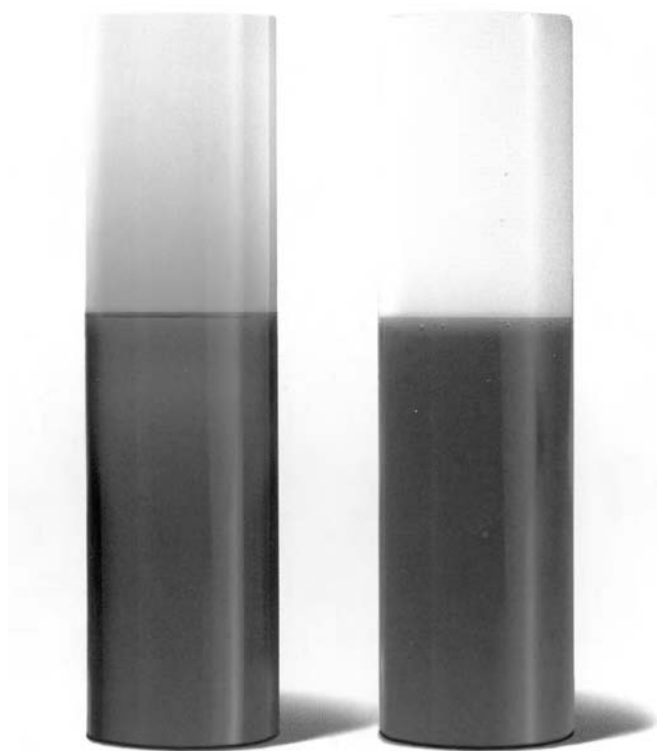
Migration of water-soluble colours can be a problem in many different food products such as layered desserts, edible ice, processed fish and decorations, where a defined line between different colours is key. The advantage of using microencapsulated products is that their coating stays intact and therefore they do not migrate as opposed to many conventional colours (Fig. 13.4). Microencapsulation also increases the brightness of many natural colours, a property that is beneficial when blending. Where many conventional colours may turn dull and grey when blended, microencapsulated products stay vivid and attractive and thereby increase the possibilities for their use. In this way food and beverage manufacturers can reduce the number of colours that they need, an advantage both in relation to storage space and for label declarations of multicoloured products, e.g., sugar confectionery.

### 13.6.2 Addition of antioxidants

The structure of carotenoids generally consists of a chain of conjugated carbon-carbon double bonds. This is the case with the commercially available colours mixed carotenoids, beta-carotene, lutein, annatto and paprika. As the double bonds can be subject to oxidation, these colours will often be formulated with different antioxidants, such as various tocopherols, ascorbic acid and rosemary extract. The antioxidants can be used singly, but are more often combined specifically to give optimal protection of a given colour. The protection applies both in the colour itself during storage, but primarily during processing and shelf life of the final food and beverage products.

With the use of antioxidants it is consequently possible to increase the stability of paprika, carotenes and lutein colours considerably and thereby match



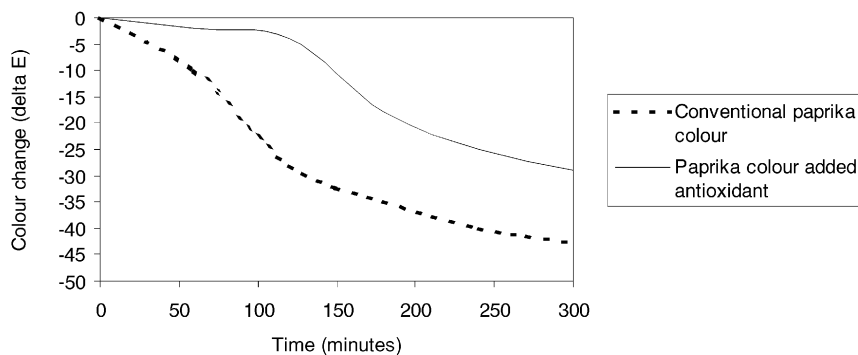


**Fig. 13.4** Jelly coloured with conventional water-soluble natural colour and encapsulated colour respectively, and topped up with yoghurt. After three days of storage the conventional colour (left) has migrated into the yoghurt layer. The encapsulated colour shows no migration after three days (right).

the shelf-life requirements in products such as soft drinks, soups, sauces and dressings, meat products and sugar confectionery. Besides the increased stability during storage, the colours are better able to withstand conditions such as high heat treatment, severe light exposure and high salt concentrations, where conventional formulations may fail (Fig. 13.5).

### 13.6.3 Emulsions

As previously mentioned, a number of natural colour pigments, e.g., carotenoids, are not soluble in water. To match the technical requirements of the primarily water-based food products, emulsions of these colours have been commercially available for a number of years. Their limitations however, can be seen in liquid products such as beverages, where they might result in settling of colour in the bottleneck, the so-called ring formation. The challenge for a natural colour manufacturer is to formulate an easy to disperse emulsion, which is stable



**Fig. 13.5** Paprika colour with and without antioxidant mixed with salt (352 CU/g fine grained salt) and exposed to light in a Heraeus accelerated lighting unit (480 W/m<sup>2</sup>, 300–800 nm). Colour change is measured using a Minolta Tristimulus CR300 calibrated with white tile.

in many different applications. This is done through a careful procedure of optimising processing conditions, choice of emulsifier and other ingredients to achieve a colour product with droplets of optimal size and surface properties.

The emulsifying agent will often be different esters or mono- and diglycerides, but also different types of gums and modified starches can be used. By controlling the homogenisation process of the emulsified colour product, the right droplet size is achieved. Small emulsion particles (below 1 µm) will give a transparent appearance in clear applications, whereas larger particles will result in a cloudy appearance. As differences in particle size may also give rise to variations in colour shade, a good process control is also essential during the manufacture of emulsion colours. At the same time this technology also gives good opportunities to create various products with different colour hues in the final application. Further requirements to the appropriate emulsifying system can also be stability at low pH as well as lack of reaction with other ingredients in the final application, e.g., alcohol in flavour formulations.

#### 13.6.4 Oil suspensions

In the same way that emulsifying or microencapsulation can be used to create a broader spectrum of natural colours for use in water-based media, it is also possible to extend the range of colours for fat-based applications with pigments that are not naturally soluble in fats and oils. This is done by milling for example carmine, red beet, carbo vegetabilis and other natural colours in oil to add pink, red, purple and black shades to the already existing possibilities within green, yellow and orange. By optimising the shape and size of the suspended particles it is possible to create colours with a very bright and vivid appearance, a high colouring ability and excellent stability. Furthermore, appropriate milling will help avoid settling during colour storage and thereby make the colour products

easier to handle. Oil suspensions produced by milling are suitable for fat coatings to enrobe ice cream, nuts and fruit as well as for cream fillings and flavoured spreads.

### 13.7 Future trends in natural colours

The natural colours market is currently growing twice as fast as that of synthetic colours. Besides the improved technological performance described in the previous section there are a number of other factors influencing this. Overall there is a general increase in demand for natural ingredients. The past 10–15 years have seen a distinct move towards naturals, especially within flavours and colours.<sup>11</sup> The move is particularly pronounced in the UK, Scandinavia and the northern part of continental Europe. Many consumers associate natural products with superior quality and a good, natural-looking colour in a food or beverage will signal high quality whilst a washed out or artificially bright product can give the opposite impression. Also, in relation to colours the fact that they are derived from well-known sources such as beetroot, grapes, cabbage and paprika, makes the consumers feel safer and thus recognition and acceptance are easier.

The general health trend will continue to be significant in the years to come. Consumers are increasingly aware of the relationship between diet and health and their own ability to influence this.<sup>12</sup> The whole functional foods concept is based on this fact and although awareness is still low in Europe, fortified food and beverage products are expected to play a continuously increasing role in mainstream diets in the years to come. Many different nutrients are today applied in functional foods and more will be added over the coming years. Some of these are natural colour pigments, which have only recently been recognised for their possible health effects.

Those pigments, that are presently acknowledged for their nutritional properties are a number of carotenoids and anthocyanins. Natural carotenoids include carotenes, lutein and lycopene and have been recognised as antioxidants that are linked to the prevention of degenerative diseases.<sup>13,14,15</sup> It is a fact that epidemiological evidence of the nutritional benefits of fruit and vegetables points to a range of carotenoids rather than a single carotenoid providing these benefits.<sup>16</sup> Anthocyanins, or polyphenols, from grape skin are known to lower the risk of cardiovascular disease in areas with a large wine consumption (the French paradox).<sup>17,18</sup>

Another consequence of the health trend is the rising interest in and demand for reassurance concerning product quality, food safety and production methods. The most visible result of this is the increase in organic products on the market, where the total organic penetration of the EU food and beverage market is expected to be 9.2% in 2004.<sup>19</sup> The demands on the natural colours industry will not only be for organic colours but also for a generally higher level of information with regard to production methods, specifically HACCP, as well as traceability of all ingredients. The industry will also be expected to continue to

respond quickly to any future food scares and make changes in formulations to match shifting market requirements.

In general, competition is increasing within the food and beverage industries and greater pressure is put on new product development. In their search to differentiate, product developers are looking at new ingredient options and natural colours are one of many possibilities. Simultaneously the natural colours industry is required continuously to bring forward new colouring opportunities to match the increasing demands of their customers. Future developments are expected to concentrate on improvements of well-known technologies within the formulation and processing of existing colour pigments. A further development of microencapsulation will particularly be in focus due to the many superior advantages of this technology.

Besides the new technological solutions, natural colour manufacturers are also looking at a number of new pigment sources. One of the limitations in developing totally new colour formulations is the lengthy and costly safety testing and regulatory approval process. Therefore, 'untapped' sources of raw materials that conform to current regulations give valuable options to develop new colour products. Recent examples of these are blade carrot, red cabbage and algae beta-carotene. A final factor that is affecting the natural colours industry is the considerable consolidation taking place. This allows a concentration of R&D capabilities and resources thereby increasing the overall output of new products.

### 13.8 Sources of further information and advice

The information given in this chapter is an overall description of the range of natural colours and how to use them in different applications. The manufacturing processes used by the food and beverage industries vary and the use of natural colours will often be a case-by-case evaluation. For the best results it is recommended to follow specific advice from the colour supplier.

A detailed description of the biochemistry, chemistry and biology of natural colour pigments can be found in the book *Natural Food Colorants*. The book also covers the role of biotechnology in the production of colorants as well as safety aspects.<sup>1</sup> Another book with the same title, *Natural Food Colorants*, assembles the manuscripts from a two-day Basic Symposium on Natural Colorants held in Chicago in July 1999. The book covers natural colorant chemistry, preparation, formulation, application and measurement as well as safety, regulatory and health considerations.<sup>20</sup>

Organisations focusing on natural colours include Natural Food Colours Association (NATCOL) and International Association of Color Manufacturers (IACM). NATCOL's purpose is to promote the use of natural colours in food, feed, cosmetics and pharmaceutical products. NATCOL offers national and international authorities and organisations expertise regarding natural colours and supports the food-processing industry by supplying it, through the individual

members, with up-to-date technical advice about natural colours ([www.natcol.org](http://www.natcol.org)).

IACM's mission is actively to represent the interests of the colour industry by assuring the safe use of all colour additives, and to promote the industry's economic growth by actively participating in technology development and regulatory and legislative issues that affect the industry worldwide ([www.iacmcolor.org](http://www.iacmcolor.org)).

More information on legislation can be found on the following web-sites:

US-legislation: [http://www.access.gpo.gov/nara/cfr/waisidx\\_01/21cfr73\\_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr73_01.html)

EU legislation: [http://europa.eu.int/comm/food/fs/sfp/addit-flavor/flav08\\_en.pdf](http://europa.eu.int/comm/food/fs/sfp/addit-flavor/flav08_en.pdf)

Australian legislation: <http://www.anzfa.gov.au/foodstandards/>

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## Calibrated colour imaging analysis of food

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### 14.1 Introduction

As outlined in Chapter 2 food total appearance properties govern expectations. Total appearance of a static object consists of visual structure, surface texture, colour and colour patterning, gloss and gloss patterning, and translucency and translucency patterning (Hutchings, 1999). In a market place heavily influenced by what we see the ability to make measurements of these individual properties will be a valuable tool for research, production and marketing.

There are many applications for good appearance measurement practice within the industry. Examples concern monitoring during storage and processing, in-field crop monitoring, colour communication, and understanding of customer behaviour. Until the present the main psychophysical measurement methods available were colour specification using colorimetry and spectrophotometry with instruments and methodology designed for flat, opaque, evenly coloured materials such as paints, plastics and textiles. Such instruments have low spatial resolution and measure average colour values only. However, most foods are characterised by their non-uniformity of colour. In general, conventional colour specification instruments, although suitable for use with food materials, are inherently unsuitable for the colour and colour uniformity measurements of many whole foods. The new technology of calibrated colour imaging analysis not only offers a methodology for specification of uneven coloration but it can also be applied to the specification of the other attributes of total appearance. The ideal instrument is one that can specify colour and other total appearance properties of non-uniformly coloured, translucent, diffusely as well as specularly reflecting, irregularly shaped, three-dimensional objects. This description fits most natural, processed and manufactured foods (Hutchings *et al.*, 2001).

## 14.2 Digital camera characterisation

The technology for specifying total appearance of food items is based upon digital cameras which can capture images in digital format quickly without film processing. The digital images can be easily processed, duplicated, modified or transmitted via a network.

Colour is specified by the spectral distributions of three elements: light source, sample and observer (CIE, 1986). Each of these was defined by the *Commission Internationale de l'Eclairage* (CIE) in terms of spectral distribution across the visible spectral range, say 400 to 700 nm. The *RGB* colour signal of a digital camera can be expressed as:

$$\begin{aligned} R &= \int_{400nm}^{700nm} S(\lambda)r(\lambda)\bar{r}(\lambda)d\lambda \\ G &= \int_{400nm}^{700nm} S(\lambda)r(\lambda)\bar{g}(\lambda)d\lambda \\ B &= \int_{400nm}^{700nm} S(\lambda)r(\lambda)\bar{b}(\lambda)d\lambda \end{aligned} \quad 14.1$$

where  $S(\lambda)$  describes the spectral power distribution (SPD) of a given source for illuminating the object and  $r(\lambda)$  is the spectral reflectance of an object. The camera's spectral sensitivities  $\bar{r}(\lambda)$ ,  $\bar{g}(\lambda)$ , and  $\bar{b}(\lambda)$  represent three types of spectral sensor. It is obvious that the colour signal produced by a digital camera relates to its sensor spectral sensitivity, which means the *R, G, B* signals generated by a camera are **device dependent**, i.e. each different camera has its own different sensor characteristics.

In the human visual system, the *X, Y* and *Z* tristimulus values are calculated from spectral power as given in the following equations (CIE, 1986).

$$\begin{aligned} X &= \int_{400nm}^{700nm} S(\lambda)r(\lambda)\bar{x}(\lambda)d\lambda \\ Y &= \int_{400nm}^{700nm} S(\lambda)r(\lambda)\bar{y}(\lambda)d\lambda \\ Z &= \int_{400nm}^{700nm} S(\lambda)r(\lambda)\bar{z}(\lambda)d\lambda \end{aligned} \quad 14.2$$

where  $\bar{x}(\lambda)$ ,  $\bar{y}(\lambda)$ , and  $\bar{z}(\lambda)$  refer to the colour matching functions of the CIE standard observer. If two colour stimuli have the same tristimulus values, they will look alike under the same viewing conditions. Hence, the tristimulus values are **device independent**.

It is clear from equations 14.1 and 14.2 that camera *RGB* values do not match the CIE *XYZ* values because the camera sensors in general do not have the same



spectral sensitivities as the CIE standard observer. This is known as **observer metamerism**.

In order to measure an object in terms of device independent colour from a digital camera, there is a need to correlate the camera *RGB* signals and CIE *XYZ* values. This is known as **camera characterisation**.

The most common technique for digital camera characterisation consists of presenting the camera with a series of colour patches in a standardised reference chart with known *XYZ* values and recording the averaged *RGB* signals for each patch. The frequently used reference charts include Macbeth ColorChecker (McCamy *et al.*, 1976), ColorChecker DC (ISO 17321, 1999a) and IT8 (ISO 12641, 1997). Polynomial fitting techniques can then be applied to interpolate the data over the full range and to generate inverse transformations. This method is known as Colorimetric Based Camera Characterisation.

Alternatively, Spectral Based Camera Characterisation can be used. This can be divided into two: measurement of the spectral sensitivities of the camera and recovery of the spectral reflectances. Both will be introduced later.

An International Organisation for Standardisation (ISO) working standard (ISO 17321, 1999a) was produced by a joint working group of the Technical Committees ISO/TC42/WG18, Photography, and ISO/TC130/WG3, Graphic Technology.

### 14.3 Colorimetrically-based camera characterisation

There are three methods for colorimetrically characterising a camera, namely:

- least-squares fitting using linear or non-linear transformation (Kang, 1992; Hong *et al.*, 2001)
- look-up table with interpolation (Hung, 1991; 1993)
- Neural Network (Kang and Anderson, 1992)

An example using the least-squares method is given here. Suppose the reference target has  $N$  colour samples. A ninth-order polynomial regression is applied to make an empirical transformation model. The equations can be written as:

$$\begin{aligned}
 X_1 &= a_{1,1}R_1 + a_{1,2}G_1 + a_{1,3}B_1 + a_{1,4}R_1^2 + a_{1,5}G_1^2 + a_{1,6}B_1^2 + a_{1,7}R_1G_1 \\
 &\quad + a_{1,8}G_1B_1 + a_{1,9}R_1B_1 \\
 X_2 &= a_{1,1}R_2 + a_{1,2}G_2 + a_{1,3}B_2 + a_{1,4}R_2^2 + a_{1,5}G_2^2 + a_{1,6}B_2^2 + a_{1,7}R_2G_2 \\
 &\quad + a_{1,8}G_2B_2 + a_{1,9}R_2B_2 \\
 X_N &= a_{1,1}R_N + a_{1,2}G_N + a_{1,3}B_N + a_{1,4}R_N^2 + a_{1,5}G_N^2 + a_{1,6}B_N^2 + a_{1,7}R_NG_N \\
 &\quad + a_{1,8}G_NB_N + a_{1,9}R_NB_N
 \end{aligned}
 \tag{14.3}$$

and similarly for  $Y$  and  $Z$ .

Here,  $R_i$ ,  $G_i$  and  $B_i$  refer to the camera's raw signals for sample  $i$  and  $a_{i,j}$  are the coefficients to be obtained for the camera characterisation model.

If we let

$$M = \begin{bmatrix} X_1 & X_2 & \dots & X_N \\ Y_1 & Y_2 & \dots & Y_N \\ Z_1 & Z_2 & \dots & Z_N \end{bmatrix}, \quad A = \begin{bmatrix} a_{1,1} & \dots & a_{1,9} \\ a_{2,1} & \dots & a_{2,9} \\ a_{3,1} & \dots & a_{3,9} \end{bmatrix},$$

$$V = \begin{bmatrix} R_1 & R_2 & \dots & R_N \\ G_1 & G_2 & \dots & G_N \\ B_1 & B_2 & \dots & B_N \\ R_1^2 & R_2^2 & \dots & R_N^2 \\ G_1^2 & G_2^2 & \dots & G_N^2 \\ B_1^2 & B_2^2 & \dots & B_N^2 \\ R_1 G_1 & R_2 G_2 & \dots & R_N G_N \\ B_1 G_1 & B_2 G_2 & \dots & B_N G_N \\ R_1 B_1 & R_2 B_2 & \dots & R_N B_N \end{bmatrix}$$

then, the coefficient matrix  $A$ , the  $XYZ$  matrix  $M$ , and the camera  $RGB$  matrix  $V$  have the relationship:

$$M = AV$$

Since  $M$  and  $V$  are known, therefore, the coefficient matrix  $A$  can be determined by the following:

$$A = MV^T(VV^T)^{-1} \quad 14.4$$

Once the coefficients in matrix  $A$  are known, the  $XYZ$  values can be calculated from the given RGC values using equation 14.5.

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = A\nu \quad 14.5$$

$$\text{Hence, } \nu^T = (R, G, B, R^2, G^2, B^2, RG, GB, RB).$$

In principle, the higher the order of polynomial used, the more accurate the colour space transformation. However, there are some important parameters to be considered: the material of the test target, the number of colours used for deriving the transform coefficients and their distribution throughout the colour space. The predicted error between the measured and predicted tristimulus values can be calculated using a colour difference formula such as CIELAB (CIE 15.2, 1986).

## 14.4 Spectral-based camera characterisation

Spectral-based camera characterisation can be further divided into two methods:

- determining the spectral sensitivities
- recovery of the surface reflectance

The former method is to directly measure the camera spectral sensitivities  $\bar{r}(\lambda)$ ,  $\bar{g}(\lambda)$  and  $\bar{b}(\lambda)$  using a monochromatic light source, say at a 10 nm interval. Each monochromatic light is measured by the camera in question. By scanning the wavelength output of the monochromatic light source across the visible spectrum and recording the camera *RGB* signals at each wavelength, the camera's responses can be measured directly. The details of this method can be found in ISO 17321-1 (1999b).

The second method is to predict the surface reflectance. The surface reflectance function  $r$  is approximated by a linear model:

$$r = \sum_{i=1}^n \sigma_i \epsilon_i. \quad 14.6$$

where  $\epsilon_i$  values are the basis functions, most commonly derived by Singular Value Decomposition or Principal Components Analysis;  $n$  is the number of basis functions used and is normally less than the number of components of the reflectance vector  $r$ . The weighting factors,  $\sigma_i$ , can be chosen according to basic *RGB* response equation and different constraints.

A number of researchers used this methodology (Cohen, 1964, 1988; D'Zmura and Lennie, 1986; Marimont and Wandell, 1992; Westland and Thomson, 2000) to predict spectral reflectance. The results are summarised as follows:

- As the dimension of the linear model increases, the approximation improves.
- A linear model with only three proper basis functions can reasonably approximate the surface reflectance of a set of samples used to optimise the model.
- A linear model that best describes any particular class of surfaces will be the data set that accounts for the greatest proportion of variance in the collection of surface reflectance functions.
- A linear model with about seven basis functions can account for over 99% of the variance in the data and the mean reconstruction errors by the linear models are less than one  $\Delta E_{ab}^*$ .

In general, too many basis functions will produce spectral reflectance functions too rugged to be realistic. Methods for overcoming this phenomenon have been only partially successful. A model with three basis functions will give the reflectance function with some smoothness property, but the reflectance function cannot be assured within the desired range (less than one and greater than zero).

Alternatively, there are other methods which can also predict spectral reflectance functions such as those based on a smoothness parameter (Li and Luo, 2001a) and a colour inconstancy index (Luo *et al.*, 1999). The DigiEye system applies these two measures to predict the spectral reflectance functions. The smoothness approach is to minimise the difference between the values in

adjacent wavelengths in a reflectance function. The colour inconstancy index (CON) is added to provide the predicted reflectance with a high degree of colour constancy, which ensures constant colour appearance across different illuminants. The combined method is described in Li and Luo (2001b).

## 14.5 The DigiEye imaging system

Using the methodologies outlined above the DigiEye imaging system based upon the digital camera was developed (Luo *et al.*, 2001). The system as shown in Fig. 14.1 includes a digital camera (1), a computer (2) with a colour sensor (3) and an illumination box (4). The computer software includes four functions: camera characterisation, colour measurement, monitor characterisation and texture profiling.

The digital camera is used to capture the image of an object that is placed in the illumination box specially designed by VeriVide Limited to illuminate the samples consistently in two alternative illuminants (D65 or A). The digital cameras used are different for different applications. For example, a lower resolution camera may be sufficient for measuring colours but a high-resolution camera will be needed for capturing high quality images with fine detail or textures. The computer is mainly used to operate the system and includes software and a driver to capture images from the digital camera. The system also includes a colour sensor used for measuring CRT colours; this ensures the display of high colour fidelity colours on screen.

The illumination box containing typically a D65 simulator provides a stable illumination environment. The sample is illuminated by two sets of lamps at 45° to the sample. For colour measurements both lamps are used but for texture measurements the sample is illuminated from one side only. This produces higher contrast. The illumination box used is critical for achieving accurate colour images because it provides a highly stable illumination environment.

The computer software includes several important functions:

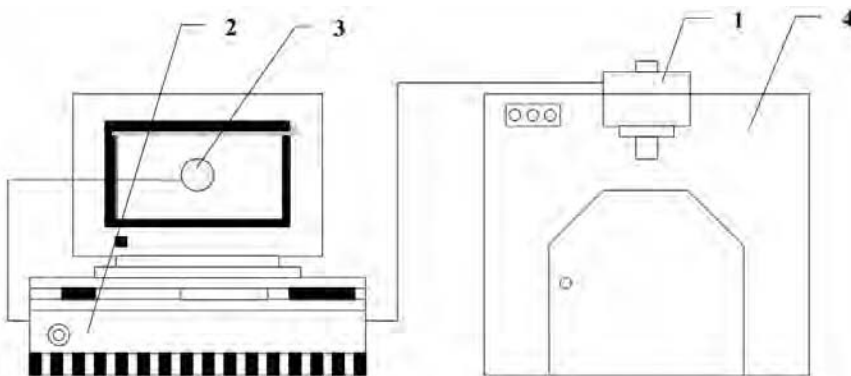


Fig. 14.1 The DigiEye, a schematic diagram.

- *camera characterisation* for transforming camera *RGB* signals to CIE specifications by means of a reference chart
- *colour measurement* for capturing colour images and for measuring the colour of each pixel or a group of pixels in the captured images – these are reported in terms of colorimetric values and spectral reflectance function
- *monitor characterisation* for calibrating displays to ensure high colour fidelity images displayed on the monitor
- *texture profiling* for building an image database to simulate the effect of applying different colours onto a given surface texture and for applying colours onto a pre-defined texture profile.

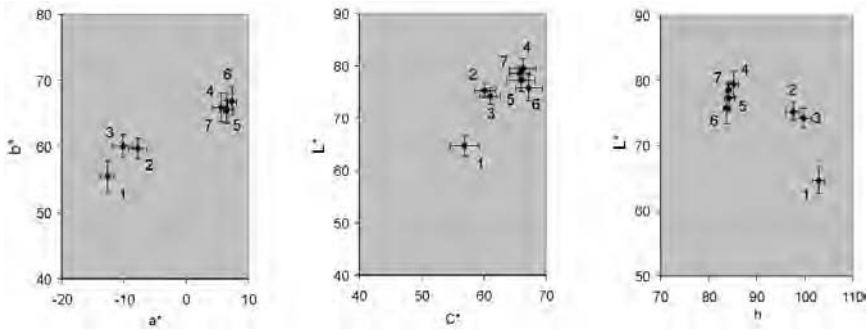
## 14.6 Applying colour imaging analysis to the measurement of particular foods

Colour imaging analysis has been applied to a number of food materials. The baking curve has been determined for a plain cracker biscuit (Hamey *et al.*, 1995). Tomatoes have been analysed for hue distribution (Choi *et al.*, 1995), and size, shape and colour (Edan *et al.*, 1994). Apples have been assessed for colour, defect, shape and size using a hue histogram and linear discriminant analysis (Varghese *et al.*, 1991). Extruded food products were characterised for colour and surface texture, the latter using fast Fourier transforms of the hue, saturation and intensity data (Tan *et al.*, 1994). Image analysis has been used in the sorting of bell peppers by statistical classification of hue (Shearer and Payne, 1990), to study colour and marbling of beef (Gerrard *et al.*, 1996), and to detect defects in potatoes using colour and shape (Grenander and Manbeck, 1993). However, use of calibrated instrumentation, such as that provided by DigiEye, has been restricted.

Three examples using calibrated equipment have been taken to illustrate the problems and potential solutions for the measurement of elements of total appearance. The first describes the appearance of ripening banana, the second the colour and structure of a breakfast cereal, the third the gloss of an orange.

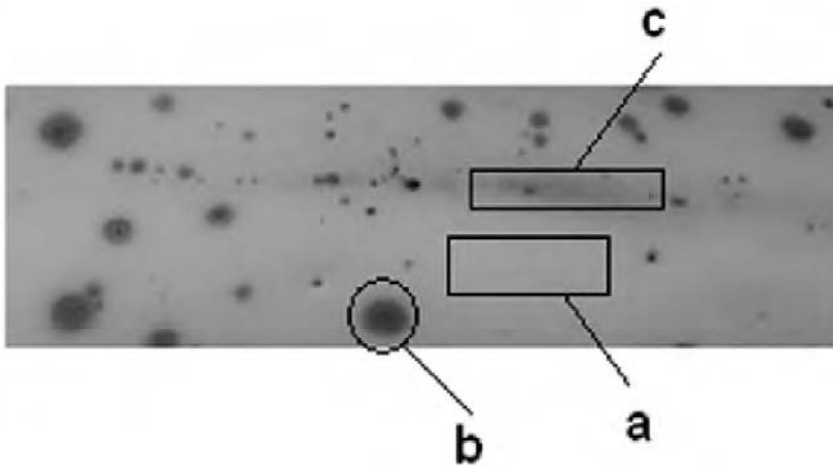
### 14.6.1 Banana

The colours present on a number of Windward Island bananas at various stages of ripening during storage at 21°C were measured. Ripening curves of the developing background colour, in terms of  $a^*/b^*$ ,  $L^*/h^*$  and  $L^*/C^*$ , are shown in Fig. 14.2. Pixel distributions are shown in terms of standard deviation bars. Samples 1, 2 and 3 were greenish increasing in visually determined lightness in the order 1,3,2. The other samples were yellowish increasing in visually determined depth of yellow, that is in decreasing  $L^*$ , in the order 4,7,5,6. The colour coordinate distributions precisely reflected these orders. Thus a three-dimensional ripening curve can be constructed. The fading of the green chlorophyll, points 1 to 3 to 2, gives way to an increasingly darker yellow, points 4 to 7 to 5 to 6.

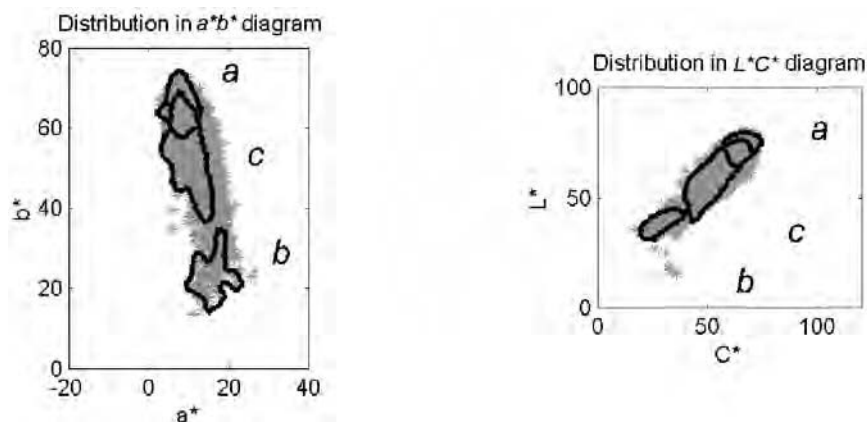


**Fig. 14.2** The banana background colour ripening curve (points 1–2 greenish, points 4–7 yellowish; see text for detailed explanation).

Over ripe samples were studied for colour uniformity. Figure 14.3 shows three areas selected for measurement. These are the yellowish background colour (a) and two areas of brown (b and c). The brown areas corresponded to the strip (c) indicating dark brown polyphenol damage to the flesh showing through the translucent skin, and the brown spots (b) appearing on the skin surface that indicate that the fruit is fully ripe. These areas can be identified and categorised in terms of the coordinate distributions shown in Fig. 14.4. Hence, specific fruits can be identified according to their colour, colour uniformity, degree of ripeness and damage. Similar conclusions are likely to be applicable to all fruits where degree of ripening is characterised by change in surface appearance. It will be possible to develop software tools for automatic classification based on such findings.



**Fig. 14.3** Ripening zones of a banana, background colour (a), external brown spots (b), brown over ripe flesh showing through the translucent skin (c).



**Fig. 14.4** Pixel distributions corresponding to different areas of the banana marked on Fig. 14.3.

#### 14.6.2 Breakfast cereal

Samples of a breakfast cereal product were oven baked at 180°C and sampled at 15-minute intervals up to a maximum of 105 minutes. Each sample exhibited a range of colours from whitish to darkish brown the degree of which increased with baking time. Images were captured, clipped from their grey background and analysed. Values of  $L^*$ ,  $a^*$  and  $b^*$  are shown in Table 14.1. As baking time increased there was an eventual fall in  $L^*$ ,  $a^*$  increased then remained approximately constant, while  $b^*$  first increased then fell. The distribution of the mid 75% of pixels is shown in a three dimensional form in Fig. 14.5a, median values being plotted in Fig. 14.5b. The changes of coordinate direction in Fig. 14.5 indicate the presence of a number of different major pigment development reactions. As cereals are heated carbohydrate/amino acid browning reactions occur during which colour and flavour develop. Caramelisation involving thermal degradation of sugars without amine participation also takes place and lipid oxidation decomposition products react with proteins. Finally there are complex pigment reactions involving the greying of natural carotenoids present in the outer layers of cereals (Dworschak, 1980). No attempt was made to identify the dominant chemical changes corresponding to the two major pigment development changes indicated by Fig. 14.5.

Table 14.1 also contains maximum and minimum values that reveal the variability of changes occurring in the cereal. Although mean values of  $L^*$  decrease by 21% over the 105 minutes baking the values for the lightest parts of the structure decreases by only 2%. This demonstrates the relative inertness of the paler parts of the structure, which are possibly caused by poorly presented starch. On the other hand the minimum value of  $L^*$  for all biscuits was always zero. These areas correspond to the gaps in the structure that therefore always appear black having near zero luminance. Similarly the minimum values of  $a^*$  and  $b^*$  were zero, no samples being greenish or bluish. Such areas of specific

**Table 14.1** Effect of baking time on breakfast cereal colour

Baking time (min)		Mean	Median	Minimum	Maximum	Standard deviation
0	L	57.15	57.97	0.00	91.94	12.90
	a	3.15	3.16	0.00	101.60	3.79
	b	23.17	23.10	0.00	60.42	5.07
15	L	56.90	57.38	0.00	91.65	13.21
	a	2.96	2.91	0.00	105.10	3.75
	b	25.36	25.18	0.00	60.09	5.31
30	L	53.54	54.19	0.00	92.46	12.44
	a	11.26	11.07	0.00	105.10	4.09
	b	30.76	30.67	0.00	58.18	6.13
45	L	52.06	52.88	0.00	92.24	12.92
	a	12.72	12.45	0.00	105.10	4.70
	b	30.53	30.54	0.00	59.26	6.59
60	L	47.20	47.57	0.00	91.87	12.30
	a	14.15	13.76	0.00	105.10	4.82
	b	28.44	27.86	0.00	59.79	6.94
75	L	46.29	47.07	0.00	90.61	12.42
	a	14.27	13.81	0.00	105.10	5.04
	b	28.17	27.62	0.00	59.96	6.99
90	L	45.31	45.82	0.00	88.54	11.91
	a	13.57	13.26	0.00	105.10	4.93
	b	26.97	26.59	0.00	57.37	6.87
105	L	45.16	45.83	0.00	90.36	12.14
	a	14.85	14.60	0.00	105.10	4.93
	b	25.60	25.24	0.00	60.46	7.19

colour can be confirmed and identified using specific colour range properties. For example, the volume of the darkest part of the structure having  $L^*$  values of less than 20 is approximately 2% of the total. Alternatively specific areas of an image can be identified visually, isolated, and analysed for colour values.

#### 14.6.3 Orange gloss

Differences in light scattering across the surface of a fruit or vegetable give rise to perceptions of gloss. Specular scattering is greater from smooth surfaces such as those of aubergine and tomato. Rougher skin, such as that possessed by the orange is less specular. Our love for shiny surfaces has led to the industry practice of applying wax coatings to fruit hence as well as reducing respiration the fruit becomes more attractive. Waxes designed to reduce gas exchange,



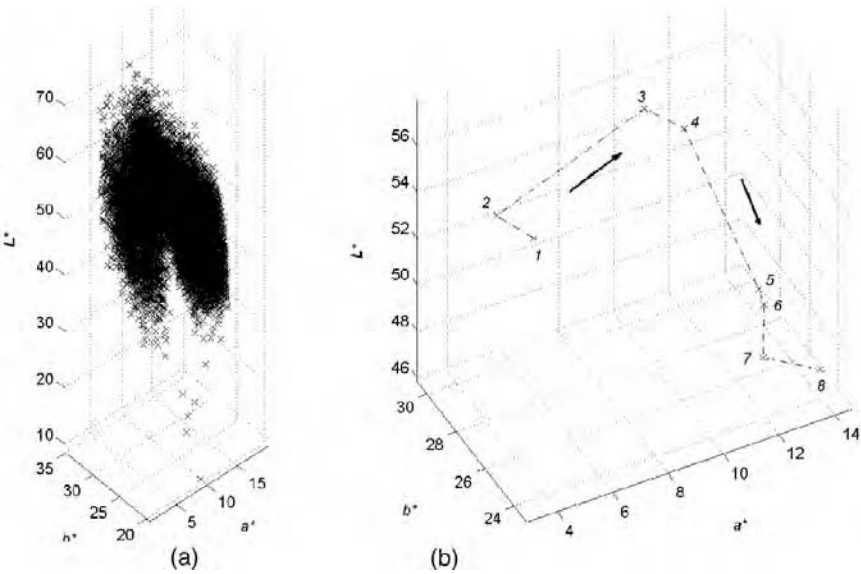


Fig. 14.5 Baking curve for breakfast cereal.

weight loss and fungal growth are also designed to be glossy. The wax fills small pits in the surface with a polishable material of similar refractive index. This reduces diffuse but increases directional light scattering and the fruit is seen to be glossier.

An unpolished orange having no artificially applied waxes was lightly polished sequentially with six small applications of beeswax. The changes in median  $L^*$ ,  $C^*$  and  $h^*$  were not dependent on polishing. However, the maximum value of  $L^*$  may be expected to be related to the perceived gloss and values are given in Table 14.2. This indicates that there was a visually significant increase in  $L^*$  on the first application of wax but none thereafter. These preliminary findings indicate that gloss could with a minimum application be increased to a maximum value. This may depend on the materials and methods used.

Table 14.2 Effect of sequential polishing on the maximum value of  $L^*$

Number of waxings	Maximum value of $L^*$
0	81.8
1	86.0
2	86.0
3	85.4
4	84.7
5	84.2
6	85.7

## **14.7 Applying colour imaging analysis to the sensory analysis of foods**

Image analysis can bring significant benefits to a number of areas of food sensory science and consumer understanding.

### **14.7.1 Construction of a food science colour order system**

Studies of the sensory aspects of food appearance are hampered by the three-dimensional nature of colour itself (Hutchings, 1999). This has prohibited the production of physical colour scales that mimic the sequences of colour changes occurring with natural pigments. An example concerns the changes taking place in chlorophylls and carotenoids during tomato ripening. Using the new technology physical colour scales can be developed with respect to the colour and pigment science of the particular food system under examination. This work could lead to the development of a colour atlas that can be used by panellists to monitor changes in biological colour easily and consistently. The above quoted studies on the changes occurring during banana ripening and cereal browning colour will contribute to the construction of such an order system. There has also been a study of the colour gamuts of freshly cooked frozen peas and of stored cooked peas (Hutchings *et al.*, 2002).

### **14.7.2 Sensory studies using calibrated monitors**

Digital imaging provides the methodology for colour communication and archiving, for example, in the textile industry. The modern food business is global and such visual link communication perhaps between grower and processor in different parts of the world could facilitate optimal crop monitoring and selection. It may also become possible to use calibrated monitors for product development and selection. Panellists would be able to view virtual products on screen avoiding the expense of sample manufacture. Preliminary work toward such an aim has been commenced (Pointer *et al.*, 2002). Calibrated image analysis is also applicable to sensory studies in the field enabling the effects of, for example, appearance make-up of products on the plate, on the pack and on the shelf to be determined. This can be done in store, in the home and in hospital.

## **14.8 Future trends**

Image analysis is highly appropriate to the food industry but work on calibrated systems is in its infancy. What has been demonstrated is the wide applicability to the business. It is necessary for the instrumentation to be applied and tuned to the understanding of factors comprising all elements of total appearance. That is, applied to the quantification and specification of visual structure, surface structure, colour, gloss, translucency but also most important for the industry

variations occurring in these properties. The needs of the industry will vary for different products and food materials but wide application to the industry's problems is certain.

## 14.9 Sources of further information and advice

The technology reported here is new. A short review of published work is contained in Hutchings (1999).

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